National Toxicology Program
U.S. Department of Health and Human Services

Center For The Evaluation Of Risks To Human Reproduction

DRAFT

NTP-CERHR EXPERT PANEL REPORT on REPRODUCTIVE and DEVELOPMENTAL TOXICITY of 2-BROMOPROPANE

OCTOBER 2001 **NTP-CENTER-XXXXX-00**

PREFACE

The NTP-CERHR is headquartered at NIEHS, Research Triangle Park, NC and is staffed and administered by scientists and support personnel at NIEHS and at Sciences International, Inc, Alexandria, Virginia.
Reports can be obtained from the website (http://cerhr.niehs.nih.gov) or from:
CERHR
Sciences, International, Inc.
1800 Diagonal Road, Suite 500
Alexandria, VA 22314-2808
Telephone: 703-838-9440

A Report of the CERHR Bromopropanes Expert Panel:

Name	Affiliation
Kim Boekelheide, MD, PhD, Chairman	Brown University, Providence, RI
Cynthia F. Bearer, MD, PhD	Case Western Reserve U., Cleveland, OH
Sally Perreault Darney, PhD	EPA, Research Triangle Park, NC
George P. Daston, PhD	Proctor & Gamble, Cincinnati, OH
Raymond M. David, PhD	Eastman Kodak Company, Rochester, NY
Ulrike Luderer, MD, PhD	University of California-Irvine, Irvine, CA
Andrew F. Olshan, PhD	University of North Carolina, Chapel Hill, NC
Wayne T. Sanderson, PhD, CIH	NIOSH, Cincinnati, OH
Calvin C. Willhite, PhD	DTSC, State of California, Berkeley, CA
Susan Woskie, PhD, CIH	University of Massachusetts, Lowell, MA

With the Support of CERHR Staff:

NTP/NIEHS

Michael Shelby, PhD	Director, CERHR
Christopher Portier, PhD	Associate Director, NTP

Sciences International, Inc.

John Moore, DVM, DABT	Principal Scientist
Annette Iannucci, MS	Toxicologist
Gloria Jahnke, DVM	Toxicologist

TABLE OF CONTENTS

PREFACE ..... **III**

A REPORT OF THE CERHR BROMOPROPANES EXPERT PANEL: ..... **V**

LIST OF TABLES ..... **IX**

LIST OF FIGURES ..... **XI**

1.0 CHEMISTRY, USAGE, AND EXPOSURE **1**

1.1 CHEMISTRY 1
1.1.1 Nomenclature 1
1.1.2 Formula and Molecular Mass 1
1.1.3 Chemical and Physical Properties 1
1.1.4 Technical Products and Impurities 1
1.2 USE AND HUMAN EXPOSURE 1
1.2.1 Production 1
1.2.2 Use 2
1.2.3 Occurrence 2
1.2.4 Human Exposure 2
1.3 UTILITY OF DATA 2
1.4 SUMMARY OF HUMAN EXPOSURE DATA 2

2.0 GENERAL TOXICOLOGY AND BIOLOGICAL PARAMETERS **3**

2.1 TOXICOKINETICS 3
2.2 GENERAL TOXICITY 4
2.2.1 Human Data 4
2.2.2 Animal Data 6
2.3 GENETIC TOXICITY 9
2.4 CARCINOGENICITY 10
2.5 SUMMARY OF GENERAL TOXICOLOGY AND BIOLOGICAL EFFECTS 10

3.0 DEVELOPMENTAL TOXICITY DATA **13**

3.1 HUMAN DATA 13
3.2 EXPERIMENTAL ANIMAL TOXICITY 13
3.3 UTILITY OF DATA 13
3.4 SUMMARY OF DEVELOPMENTAL TOXICITY 13

4.0 REPRODUCTIVE TOXICITY **14**

4.1 HUMAN DATA 14
4.2 EXPERIMENTAL ANIMAL TOXICITY 17
4.2.1 Female Reproductive Toxicity 17
4.2.2 Male Reproductive Toxicity 23
4.2.3 Mechanisms of Reproductive Toxicity 34
4.3 UTILITY OF DATA 34
4.4 SUMMARY OF REPRODUCTIVE TOXICITY 34

5.0 SUMMARIES, CONCLUSIONS, AND CRITICAL DATA NEEDS 38

5.1 SUMMARY AND CONCLUSIONS OF REPRODUCTIVE AND DEVELOPMENTAL HAZARDS 38
5.2 SUMMARY OF HUMAN EXPOSURE 38
5.3 OVERALL CONCLUSIONS 38
5.4 CRITICAL DATA NEEDS 38

6.0 REFERENCES 39

LIST OF TABLES

Table 1-1. Chemical and Physical Properties of 2-BP....1
Table 2-1. Summary of General Toxicity Inhalation Studies in Male Rats....11
Table 4-1. Major Effects in Wistar Rats in Reproductive Toxicity Study by Kamijima et al. (1997) ..17
Table 4-2. Major Effects in Reproductive Toxicity Study in Wistar Rats by Yu et al. (1999)....19
Table 4-3. Major Effects in Reproductive Toxicity Study in Sprague-Dawley Rats by Lim et al. (1997)....21
Table 4-4. Reproductive Toxicity Study in ICR mice by Sekiguchi and Honma (1998). 22
Table 4-5. Reproductive Toxicity Study in Wistar Rats by Ichihara et al. (1997)....24
Table 4-6. Major Effects in Reproductive Toxicity Study in Sprague-Dawley Rats by Yu et al. (1997). 26
Table 4-7. Major Effects in Reproductive Toxicity Study in Sprague-Dawley Rats by Wu et al. (1999a). 28
Table 4-8. Effect on germ cells numbers in Omura et al. (1997a,b) study. 29
Table 4-9. Time-dependent reductions in Spermatogonia numbers observed by Omura et al. (1999). 30
Table 4-10. Histological analysis in Son et al (1999) study. 31
Table 4–11. Summary of Reproductive Toxicity in Inhalation Studies in Female Rats....34
Table 4-12. Summary of Reproductive Toxicity in Inhalation Studies in Male Rats 36

List of Figures

Figure 1-1. Chemical structure of 2-BP 1

1.0 CHEMISTRY, USAGE, AND EXPOSURE

1.1 Chemistry

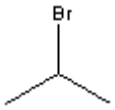
1.1.1 Nomenclature

2-Bromopropane (2-BP): CAS=75-26-3

Synonym: Isopropyl Bromide

1.1.2 Formula and Molecular Mass

Figure 1-1. Chemical structure of 2-BP



C_3H_7Br mw=123

1.1.3 Chemical and Physical Properties

Table 1-1. Chemical and Physical Properties of 2-BP.

Property	Value
Boiling Point	59.38 C at 760 mm Hg
Melting Point	-89 C
Specific Gravity	1.31 at 20 C
Solubility in Water	3,180 mg/L at 20 C
Vapor Pressure	216.47 mm Hg at 25 C
Stability	Stable with normal use and storage*
Reactivity	Incompatible with oxidizing agents.*
Log K_{ow}	2.14

Rev. in HSDB (2001), *Mallinckrodt Baker (1999)

1.1.4 Technical Products and Impurities

Two studies describe the composition of 2-BP that was used at plants in Asia. In a Korean plant, the purity of 2-BP used was 97.4% and contaminants included n-heptane (0.33%), 1,2-dibromopropane (0.2%), and 1,1,1-trichloroethane (0.01%) (Kim et al., 1996a; Park et al., 1997; Kim et al., 1999). The reported purity of 2-BP used in a Chinese plant was 98.08% and contaminants consisted of 2-propanol (1.76%), dibromopropane (0.085%), benzene (0.055%), and trichloroethylene (0.10%) (Ichihara et al., 1999). In the majority of animal studies described in Sections 2, 3, and 4, the purity of 2-BP was at least 99%.

1.2 Use and Human Exposure

1.2.1 Production

2-BP is manufactured by heating isopropyl alcohol together with hydrogen bromide (HSDB, 2001). Information about U.S production of 2-BP was contradicting. HSDB (2001) reported that Great Lakes Chemical is a producer of 2-BP. However, an OSHA report (1999), stated that 2-BP is not intentionally

produced for commercial use in the U.S., but is a contaminant of 1-BP at concentrations of 0.1-0.2%. ASTM Standards for vapor-degreasing and general grade 1-BP list 2-BP as a contaminant at a maximum of 0.1% by weight (ASTM, 2000).

1.2.2 Use

2-BP is used as an intermediate in the synthesis of pharmaceuticals, dyes, and other organics (HSDB 2001). In Asia 2-BP has been used as a replacement for chlorofluorocarbons and 1,1,1-trichloroethane (Ichihara et al., 1999; Kim et al., 1996a; Park et al., 1997; Kim et al., 1999).

1.2.3 Occurrence

No information was found that indicates if the public could be exposed 2-BP through contact with air, drinking water, food, or consumer products.

1.2.4 Human Exposure

NIOSH measured 2-BP levels in the breathing zones of workers in a plant where a 1-BP-containing spray adhesive was used in the manufacture of seat cushions (Reh, 2000). 2-BP is a contaminant found in 1-BP at 0.1–0.2%. Time weighted average (TWA) exposures to 2-BP in 16 workers ranged from 0.08–0.68 ppm with a mean of 0.28 ppm. NIOSH also measured personal exposure to 2-BP in a plant where 1-BP was used as a cold vapor degreaser in the presence of a local exhaust system (Reh and Nemhauser, 2000). 2-BP exposures in 20 employees working near the degreaser were all below the minimum detectable concentration of 0.0004 ppm.

Exposures to 2-BP were simulated in a Korean plant where 2-BP was used as a cleaning solution within a ventilation hood; area exposures were estimated at 9.2–19.6 ppm (mean 12.4 ppm) outside the exhaust hood with occasional short term exposures to 4,140.7 ppm within the exhaust hood (Kim et al., 1996a; Park et al., 1997; Kim et al. 1999). In a Chinese chemical plant, personal exposures to 2-BP exceeded the detection limit (0.2 ppm) in 12 of 14 women and 4 of 11 men and ranged from 0.88–16.8 ppm in women and 0.80–5.84 ppm in men (Ichihara et al. 1999).

1.3 Utility of Data

1.4 Summary of Human Exposure Data

In the US, 2-BP may be used as an intermediate in the synthesis of pharmaceuticals, dyes, and other organic compounds (HSDB, 2001). 2-BP may be present as a contaminant in 1-BP at 0.1-0.2% (ASTM, 2000; OSHA, 1999). No information was found that documents exposure of the public to 2-BP through contact with air, drinking water, food, or consumer products. 2-BP levels were measured in a limited number of occupational settings where 1-BP was used. Time weighted average (TWA) exposures of 0.08-0.68 ppm 2-BP were measured in the breathing zones of 16 workers using a 1-BP-containing spray adhesive (Reh, 2000). At a plant where 1-BP was used as a vapor degreaser within a local exhaust system, 2-BP exposures were below the minimum detectable concentration of 0.0004 ppm in 20 workers (Reh and Nemhauser, 2000). There are reports of occupational exposure and disease in workers involved in 2-BP production and use in Korea and Japan. These findings are discussed in Sections 2 and 4 of this report.

2.0 GENERAL TOXICOLOGY AND BIOLOGICAL PARAMETERS

2.1 Toxicokinetics

Human Data

Studies providing information on absorption and distribution of 2-BP were not identified. One study by Kawai et al. (1997) may provide limited information about the metabolism and elimination of 2-BP in humans.

Kawai et al. (1997) attempted to develop a system of biomonitoring for 2-BP based on a postulated metabolic pathway for 2-BP. They noted studies demonstrating that methyl bromide can by hydrolyzed to bromide ion and methanol *in vivo* and that a similar reaction takes place with ethylene dibromide. It was therefore postulated that 2-BP would be hydrolyzed to isopropyl alcohol which is known to be oxidized to acetone. Urinary levels of 2-BP, bromide ion, acetone, and isopropyl alcohol were measured in 5 male Japanese workers exposed to 2-BP (mean area concentration of 3 mg/m^3 [0.6 ppm]) and the values were compared to 20 unexposed males. Only the foreman, who was thought to be exposed to the highest level of 2-BP, had urinary levels of acetone and bromide that exceeded the levels found in unexposed controls. 2-BP and isopropyl alcohol were not detected in the urine of workers or non-exposed controls. Kawai et al. (1997) also examined the metabolism of 2-BP in rats; that portion of the study is described below under the animal data section.

Strength/Weaknesses: The methods for trapping and measuring 2-BP vapor concentrations, and for measuring 2-BP metabolite concentrations (isopropanol, acetone, bromide, parent) in urine were found to be linear over a reasonably wide dynamic range. The rat experiment used to establish the linearity of the urinary assay indicated that parent and isopropanol are not detectable in urine, suggesting that metabolism of parent is complete, as is oxidation of isopropanol to acetone. The biomonitoring of 5 workers exposed to a mean concentration of 3 mg/m^3 suggest that this concentration did not increase the urinary Br or acetone level above background except in the worker believed to be most highly exposed. It should be noted however, that when these metabolite concentrations were normalized to urinary creatinine the values were within the range of unexposed controls. The strengths of the study are that the methods are reliable and the urinary monitoring permits an estimation of exposure from multiple routes. The weakness is that data are from workers exposed to comparatively low levels of 2-BP and are limited.

Utility (Adequacy) for CERHR Evaluation Process: The utility of this paper is that it provides confirmation of a metabolic pathway for 2-BP. Unfortunately, there is too little data to be able to link the metabolite biomarkers to exposure levels. The low exposure level in the study is considerably lower than the vapor concentration associated with adverse effects.

Animal Data

Studies examining the absorption and distribution of 2-BP in animals were not identified. Two studies in rats and an in vitro study provide some information on the metabolism and elimination of 2-BP.

A study by Kawai et al. (1997) to develop biological monitoring for 2-BP exposure provides some information about the metabolism of 2-BP. Sixteen female Wistar rats/group [200 g, age not specified] were exposed to 0, 500, 1,000, or 1,500 mg/m^3 [99, 199, or 298 ppm] 2-BP [purity not specified] for 4 hours. 2-BP concentrations in exposure chambers were monitored. Urine samples were collected during the 4 hours of exposure and during the 4 hours following exposure. Gas chromatography was used to

analyze the urine sample for 2-BP, acetone, isopropyl alcohol, and bromide ion. Data were analyzed by Student's unpaired t-test. Dose related and statistically significant increases were observed for 2-BP during exposure (≥500 mg/m^3), acetone during (≥500 mg/m^3) and after exposure (≥1,000 mg/m^3), and bromide ion after exposure (≥1,000 mg/m^3). The authors were not certain if 2-BP in urine during exposure resulted from direct contact of urine with the 2-BP vapors in air. The results of the experiment supported the theory that 2-BP is hydrolyzed to isopropyl alcohol and bromide ion, followed by oxidation of isopropyl alcohol to acetone, and excretion of acetone and bromide ion through urine.

Strength/Weaknesses: Described above under the human data section.

Utility (Adequacy) for CERHR Evaluation Process: Described above under the human data section.

Barnsley et al. (1966) fed 2 male rats (age and strain unspecified) a diet containing ^{35}S-labelled yeast for 3 days, injected 2 of the rats subcutaneously with 0.7 ml of 40% w/v solution of 2-BP [purity not specified] in arachis oil on the fourth day, collected urine for 24 hours following treatment, and measured metabolites in urine by radiochromatography. No significant levels of sulfur-containing metabolites were present in the urine at detectable levels.

Strength/Weaknesses: This paper may represent the state of the art in studying glutathione conjugation reactions in the early 1960s, but is of little use for risk assessment. The only thing that can be tentatively concluded from the work is that no mercapturic acid or other S-containing conjugates of 2-BP were present in the urine of two rats dosed with 2-BP and fed a diet containing 35S-labeled yeast. This provides negligible information about 2-BP metabolism or kinetics.

Utility (Adequacy) for CERHR Evaluation Process: This study is not of use for evaluating 2-BP risks.

Kaneko et al. (1997) studied the *in vitro* metabolism of 2-BP in hepatic microsomes of male Wistar rats by measuring the rate of substrate disappearance and rate of product (isopropyl alcohol) formation. The authors demonstrated that there were more than 2 sets of Vmax and Km metabolic constants. According to the authors, differences in rate between substance disappearance and isopropyl alcohol formation suggested the possibility of alternate pathways besides metabolism of 2-BP to isopropyl alcohol or that isopropyl alcohol is further metabolized. The procedures and results for this experiment were reported in the form of a short communication.

Strength/Weaknesses: This paper provides partition coefficients that may be useful in the eventual construction of a PBPK model for 2-BP. The limited metabolism data in rat microsomes suggest multiple metabolic routes, but metabolism is not otherwise characterized.

Utility (Adequacy) for CERHR Evaluation Process: This work will only have utility as the source of data for constructing PBPK models.

2.2 General Toxicity

2.2.1 Human Data

In 1995, the National Institute of Occupational Health, Korea Industrial Safety Corporation conducted an investigation in the tactile switch assembly section of a plant where a cluster of secondary amenorrhea was reported (Kim et al., 1996a; Park et al., 1997; Kim et al., 1999). Twenty-five women and 8 men, aged 20-44 years, were employed in that part of the plant and worked 12 hour shifts. The workers were involved in a process where tactile switch parts were dipped in baths of cleaning solution located within ventilation hoods. Prior to 1994, there were 2 temporary baths without ventilation hoods. In addition to

inhalation exposures, some workers were exposed dermally when they occasionally dipped their bare hands into the cleaning solution. No personal protective equipment was used by workers. A limited number of female workers were subjected to short term exposure as they fixed problems occurring underneath the hood. Eighteen months prior to the investigation, a CFC-based cleaning solution was replaced with a solution consisting of 97.4% 2-bromopropane (2-BP) with smaller percentages of n-heptane (0.33%), 1,2-dibromopropane (0.2%), and 1,1,1-trichloroethane (0.01%). Solvent concentrations in air were not measured during actual plant operations so exposures were estimated by obtaining 14 area samples under a simulated manufacturing scenario. 2-BP levels outside the hood ranged from 9.2-19.6 ppm with a mean of 12.4 ppm. The short-term concentration of 2-BP inside the hood was measured at 4140.7 ppm and the n-heptane level was 29.8 ppm. Effects in the 2-BP exposed group were compared to a control group of 65 females and 12 males who worked in another room of the same plant. Reproductive effects were a dominant finding in the 2-BP exposed group and are discussed in Section 4. Eight women who were suffering from amenorrhea also experienced pancytopenia. Mild anemia or leukopenia were observed in the other women. A bone marrow biopsy conducted in two women with marked pancytopenia revealed hypoplastic marrow. Those 2 women complained that they bruised easily. Mild pancytopenia was reported in 1 male worker who was also azoospermic but was not reported in males with oligospermia. Blood disorders were subsequently reported to be transient (Kim et al., 1999). Symptoms such as headache, dizziness, or weakness were reported by many workers. In males and females, clinical tests revealed no effects on blood clotting, kidney and liver function (except for one male), or thyroid function. Chest x-rays, urinalysis, and electrocardiographs (EKG) were also normal. Numbness and paralysis of hand muscles were subsequently reported by the workers (Yu et al., 1999a). Additional details are not available in a report written in English.

Strength/Weaknesses: These papers describe the cluster of health effects associated with 2-BP. They do a good job of narrowing down the type of work associated with the adverse effect cluster and provide good circumstantial evidence of the involvement of 2-BP in the toxicity. Clinical evaluations of reproductive and hematological effects are adequate and provide some clues about mode of action. The vapor concentrations of 2-BP in the work area were simulated, but a detailed exposure scenario is not available. Only area samples were measured and the duration of short term exposures is not known. More importantly the simulated conditions may not have replicated actual exposures that occurred when two unventilated cleaning tanks were present in the area prior to February through November of 1994. Only qualitative information about dermal exposure (it occurred) is given.

Utility (Adequacy) for CERHR Evaluation Process: These papers provide a good description of the human hazard potential of dermal/inhalation exposure to relatively high levels of 2-BP in an occupational setting. There is insufficient data for dose-response assessment.

Ichihara et al. (1999) examined reproductive and hematological effects in workers of a Chinese 2-bromopropane (2-BP) plant in order to obtain information about dose response relationships. Reproductive findings and complete study details are provided in Section 4. Personal air samples were measured in 14 women (age 24-54 years) and 11 males (age 31-56 years) who worked 8 hours/day, 5 days/week and were employed at the plant from 5-69 months. Levels of 2-BP exceeded the detection limit (0.2 ppm) in 4 men and 12 women; levels ranged from 0.80-5.84 ppm and 0.88-16.18 ppm in men and women respectively. Five female operators had slight anemia as indicated by red blood cell (RBC), hemoglobin (Hb), or hematocrit (Ht) values; exposures in those operators ranged from 5.80–10.74 ppm. In a comparison of accountants with normal menstrual cycles (exposure=<0.2-0.88 ppm; age 26-34 years), operators with normal menstrual cycles (exposure=4.09-8.60 ppm; age 25-40 years) and operators with amenorrhea or polymenorrhea (exposure=4.14-16.18 ppm; age 39-54 years), it was found that Hb, Ht, and white blood cell (WBC) levels were lower in operators with normal cycles compared to accountants. However, levels of Hb, Ht, and WBC levels in operators with amenorrhea or polymenorrhea did not differ from those of accountants but were significantly higher than levels in female operators with

normal menstrual cycles. Regression analysis revealed significant relationships between TWA and RBC, HT, and Hb level in women. A significant inverse relationship between TWA x duration of employment and Hb and Ht levels was observed. Lower concentrations of RBCs, Hb, and Ht were observed in 2 males exposed to the highest concentrations of 2-BP (1.20 and 5.84 ppm). However, regression analysis revealed no significant relationship between male hematological indices and TWA or TWA x duration of employment. The authors concluded that severe hematopoietic disorders were not observed but that a possible adverse effect on hematopoiesis following exposure to less than 10 ppm 2-BP could not be disproved. Authors stated that additional studies are needed to characterize the toxicity of 2-BP.

Strength/Weaknesses: Appropriate hematological and reproductive clinical measurements were made of workers in a 2-BP production plant. Vapor concentrations associated with each task in the synthesis, processing and storage of the 2-BP were measured. The authors expressed concern that these concentrations, measured in December in a plant that did not have an air-handling system, may not have been representative of concentrations of the volatile material in warmer months. In the results section, the authors describe 2-BP concentrations for workers doing various tasks and many of the concentrations are higher than the TWA concentrations that are listed in the tables of the study. However, the authors do not describe how those values were obtained; for example, it is not known if those values represent short term measurements for a particular task. Hematological effects were correlated with higher exposures to 2-BP. There did not appear to be effects at concentrations lower than 10 ppm, but the small size of the study makes it difficult to draw definitive conclusions.

Utility (Adequacy) for CERHR Evaluation Process: The report supports the Korean reports of adverse hematological effects of 2-BP. The demographics of the workforce, particularly the fact that only older women (age 40-50) had menstrual problems makes this study less useful in confirming ovarian effects. The airborne concentration measurements of 2-BP support a dose-response relationship, but the data are inadequate to support the dose-response analysis phase of risk assessment. In addition, regression analysis with such small number of subjects, few exposure measurements, and narrow range of mean exposure concentrations (0.9-16 ppm) is misleading. Lastly, there is no mention of short term exposure monitoring. Quantitative data describing the frequency and duration of short-term, high exposure would have been useful for determining if adverse effects are related to peak exposures.

2.2.2 Animal Data

Kim et al. (1996b) conducted an acute LC_{50} study of 2-BP in 8–9-week-old ICR mice (from Daehan Experimental Animal Center). Three mice/sex/group inhaled 0, 25,000, 30,000, 32,000, 33,000, or 35,000 ppm 2-BP (99.01% purity; chamber concentrations monitored) for 4 hour and were observed for 14 days. An LC_{50} of 31,171 ppm was estimated using a dose-mortality curve at a 95% confidence level. The LC_{100} was >32,905 ppm and the lowest lethal concentration was < 29,528 ppm.

Strength/Weaknesses: The report describes an LC50 determination in mice. The work was adequately done and the calculated LC50 appears to be reliable.

Utility (Adequacy) for CERHR Evaluation Process: This study provides hazard data for acute toxicity of 2-BP.

Ichihara et al. (1997) conducted a study to determine the testicular and hematopoietic toxicity of 2-BP in 13-week-old Wistar rats (from Shizuoka Laboratory Animal Center). Additional details about the examination of bone marrow in this study were reported by Nakajima et al. (1997a; 1997b). This section covers systemic parameters while reproductive findings and complete study details are discussed in Section 4. The experimental protocol had nine male rats/group exposed by inhalation to 0, 300, 1,000, or 3,000 ppm (1,509, 5,031, or 15,092 mg/m^3) 2-BP for 8 hours/day, 7 days/week for 9 weeks. Excessive toxicity in the high dose group led to termination of exposure after 9-11 days. Three rats in this high dose

group were sacrificed immediately after exposure; the remaining 6 rats were exposed to filtered air for the remainder of the 9 week study. Non-reproductive organs that were weighed and preserved in 10% formalin included the liver and kidneys. All treated rats experienced a dose-dependent reduction in body weight gain. Rats in the 3,000 ppm group began to recover body weight after 2-BP exposure ended and body weights at the end of the study were equivalent to the 300 ppm group. There were no histopathological findings or effects on relative liver and kidney weights at 300 and 1,000 ppm. Changes in red blood cell numbers were indicative of macrocytic anemia according to the study authors. Significant changes in hematological parameters included reductions in erythrocyte numbers (≥300 ppm), hemoglobin (1,000 ppm), platelets (300 and 1,000 ppm), and leukocytes (1,000 ppm). Histological examinations revealed hypocellular and fatty bone marrow in rats exposed to 1,000 or 3,000 ppm that was characterized by dose-related increases in adipose cells and reductions in megakaryocytes (Nakajima et al., 1997a; Nakajima et al., 1997b). There was only slight recovery of adipose cell and megakaryocyte numbers in the 6 rats of the 3,000 ppm group that were exposed to air for about the last seven weeks of the study; the cell numbers did not reach levels equivalent to those observed in lower dose groups. There were no changes in the ratio of granulocytes to erythrocytes at any dose.

Strength/Weaknesses: The study was a subchronic inhalation study in male rats, with exposures 8 hours/day, 7 days/week for 9 weeks. The number of animals per group, nine, was close to the expected number of ten for a guideline study. There were three treatment groups but the highest concentration was excessively toxic; therefore, three of these animals were sacrificed in extremis 11 days into the dosing period and the other six were taken off treatment for the remainder of the 9 weeks. The results support the toxicity of 2-BP to hematological and male reproductive systems, with no NOAEC identified. While this study is not a complete subchronic toxicity protocol, the examination of the male reproductive systems and hematological parameters was as or more thorough as the typical subchronic study.

Utility (Adequacy) for CERHR Evaluation Process: This study helps characterize the hazard potential of 2-BP to the blood and male reproductive system. It provides support that the effects observed in the human cluster studies are indeed attributable to 2-BP exposure. The data are useful for dose-response assessment, although the lack of NOAEC should be compensated for by the calculation of a benchmark concentration.

Yu et al. (1997) conducted a 2-BP toxicity study in rats to verify that adverse effects in the hematopoietic and reproductive systems of workers of a Korean electronics plant were due to 2-BP exposure (Kim et al., 1996a). Ten male Sprague-Dawley rats/group (~12 weeks old; from Daehan Animal Center) were injected intraperitoneally (IP) with 0, 125, 250, or 500 mg/kg bw 2-BP in olive oil, 6 times/week for 4 weeks. The authors acknowledged that the administration route does not pertain to occupational exposures, but stated that inhalation tests are required only if negative results are obtained with IP exposure. Data were evaluated by 2-way ANOVA and Duncan's multiple range test. This summary describes the non-reproductive effects while reproductive findings and complete study details are discussed in Section 4. Clinical signs included dizziness and lethargy 15 minutes after dosing in all treated animals. Bodyweight gain and terminal body weight were significantly lower in rats exposed to 250 and 500 mg/kg bw. Significant dose-related increases in relative organ weights were observed for the adrenals of rats exposed to 250 mg/kg bw and higher and for the lungs, spleen, liver, and brain at the highest dose (500 mg/kg bw). A histological evaluation of kidneys, liver, and pituitary revealed no distinct histopathology. Hematological evaluation demonstrated significant reductions in white blood cell count, lymphocyte count, hemoglobin concentration, and mean platelet volume in the 500 mg/kg bw group. Authors also noted a dose-related trend for reductions in granulocytes and monocytes. The only significant dose-related effects observed in the blood chemistry analysis were reduced alkaline phosphatase activity and increased cholesterol in the 500 mg/kg bw/day group.

Strength/Weaknesses: This study was a 28-day subchronic toxicity study in male rats, with 2-BP being given ip 6 days per week. The analysis included body weights, organ weights, hematologic and reproductive parameters. The results confirm the hematological and male reproductive effects. The route of administration makes these data dubious for anything other than qualitative support of hazard. One potential concern are the high hematocrit and red cell volumes in the lowest dose BP group. The values are unusually high and may signify a possible methodological problem.

Utility (Adequacy) for CERHR Evaluation Process: The study is useful as part of the weight of the evidence of hazard to male reproductive and hematopoietic systems. The use of an irrelevant route limits further use in risk assessment.

Because Korean workers exposed to 2-BP complained of numbness and paralysis in hand muscles, Yu et al. (1999a; 2001a) studied the neurological effects of 2-BP in rats. Nine, 10-week-old male Wistar rats/group (from Shizuoka Laboratory Animal Center) were exposed to filtered air or 100 or 1000 ppm (503 or 5,031 mg/m^3) 2-BP vapors (99.4% purity) for 8 hours/day, 7 days/week, for 12 weeks. [No rational was provided for dose selection.] Chamber concentrations were monitored. Neurological function was tested at week 0, 4, 8, and 12 by measuring motor nerve conduction velocity (MCV) and distal latency (DL). Parameters evaluated at sacrifice included blood chemistry, organ weight measurement, and histopathology in an unspecified number of rats. Hematological analysis were conducted in 8 rats/group and the nervous system of 1 rat/group was examined histologically. Data were analyzed by ANOVA followed by the Tukey-Kramer multiple comparison method. None of the parameters examined were affected at 100 ppm. Clinical signs of neurotoxicity, such as changes in lacrimation, salivation, pupillary response, reaction to stimuli, alertness, or pain perception, convulsions, tremors, or abnormal movements, were not observed. However, MCV was significantly reduced at week 8, and DL was significantly prolonged at weeks 8 and 12 in the 1000 ppm group. Histological examination revealed a scattered ball-like swelling in the myelin sheath of the common peroneal nerves of the tibia, but no effects were noted in spinal cord or brain. Bodyweight gain and absolute weight of brain, liver, and kidney were significantly reduced in rats of the 1000 ppm group. Histological findings for liver and kidney were not reported. Testicular histopathology is discussed in section 4.2.2. Hematological effects included significant reductions in erythrocytes, platelets, and leukocytes. Blood chemistry parameters (liver enzymes, glucose, protein, blood urea nitrogen, lipids, and electrolytes) were not affected. Authors concluded that long-term exposure to 1000 ppm 2-BP could lead to peripheral neuropathy.

Strength/Weaknesses: This study involved inhalation exposure 8 hrs/d, 7 d/wk, for 12 weeks of male rats to 100 or 1000 ppm 2-BP. The number of animals per group was 9, reasonable for this type of experiment, and the inhalation exposures were controlled adequately. There were progressive effects on peripheral nerve function, evaluated by repeated measures of the same animals. There was some histological evidence of myelin irregularities of peripheral nerves. The NOAEC was 100 ppm. There were no apparent CNS effects. It was not clear whether the data presented in Yu et al. (2001a) is from the same experiment as Yu et al. (1999a) or is a replicate experiment. The means and standard deviations for the achieved air concentrations of 2-BP are identical for the two reports, with the exception that the earlier paper reports two decimal places, the latter, one.

Utility (Adequacy) for CERHR Evaluation Process: This study identifies another potential toxic effect of 2-BP and provides data that could be used in dose-response assessment.

Zhao et al. (1999) conducted a study to compare the neurotoxicity of 2-BP, 1-BP, and 2,5-hexanedione (2,5-HD). Seven to nine, male Wistar rats/group (age not specified; from Seiwa Experimental Animal Institute) were injected s.c. with each chemical in olive oil 1 time/day, 5 days/week, for 4 weeks. Doses administered were 1.1, 3.7, or 11.0 mmol/kg bw 2-BP; 3.7 or 11 mmol/kg bw 1-BP; and 2.6 mmol/kg bw

2,5-HD. Purity of all chemicals was >97%. A control group of 9 rats was injected with the olive oil vehicle. According to the study authors, doses of 1.1, 3.7, and 11 mmol/kg bw are supposedly equivalent to doses received by inhalation of 100, 300, and 1,000 ppm BP. Bodyweights were measured weakly and maximum motor conduction velocities (MCV) and motor latency (ML) were measured every two weeks. Data were analyzed by one-way ANOVA followed by Duncan's multiple range test. Body weight gain in the 11 mmol 2-BP/kg bw group was lower compared to the control group. By 2 weeks of exposure the MCV began decreasing in treated rats and reached statistical significance in the 3.7 and 11 mmol 2-BP/kg bw groups at week 4. Dose-and time-related increases in ML occurred but were not statistically significant. All three compounds, 2-BP, 1-BP, and 2,5-hexanedione, produced qualitatively similar responses in MCV and ML. The authors concluded that 2-BP and 1-BP were equally potent and that both compounds were less potent than 2,5-hexanedione.

Strength/Weaknesses: This study evaluated peripheral nerve function in rats given 2-BP by sc injection on a 5 day/wk basis for 4 weeks. They reported effects on maximum conduction velocity. These results appear to support those of Yu et al. (1999), but the lack of relevance of the route and the limited number of animals per group (7 for all but the high dose of 2-BP) are a drawback to this study.

Utility (Adequacy) for CERHR Evaluation Process: This study confirms the hazard of 2-BP to the peripheral nervous system. The irrelevant route of exposure, small sample size and limited measurements limit its usefulness for quantitative risk assessment.

2.3 Genetic Toxicity

Maeng and Yu (1997) examined the mutagenicity and clastogenicity of 2-BP. A reverse mutation assay was conducted in *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and *E. coli* strain WP2 *uvrA*. Five different 2-BP concentrations were tested in duplicate in a preliminary (50-5,000 μg/plate) and second (313-5000 μg/plate) assay with and without S9 metabolic activation. DMSO was used as a negative control and positive controls included 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide, sodium azide, 9-aminoacridine, and 2-aminoanthracene. A dose-related increase in mutations was noted with 2-BP treatment in strain TA100 with S9 activation and in TA1535 with and without activation. Results in *E. coli* and the other *Salmonella* strains were negative. The authors stated that mutation in strains TA100 and TA1535 indicate base-pair substitutions.

For the *in vitro* chromosomal assay, Maeng and Yu (1997) treated Chinese hamster lung (CHL) cells in duplicate for 24 hours with 6 concentrations of 2-BP ranging from 0.077-2.46 mg/ml without metabolic activation and for 6 hours with S9 metabolic activation. Doses were based on a preliminary study that demonstrated growth inhibition with 2-BP treatment at 4.92 mg/ml. The negative control was DMSO and positive controls were mitomycin C and cyclophosphamide. 2-BP treatment did not produce chromosomal aberrations in the presence or absence of metabolic activation.

Maeng and Yu (1997) conducted a micronucleus assay by injecting 10 Sprague-Dawley rats/sex/group (from Dae-Han Experimental animal Center) I.P. with 0, 125, 250, or 500 mg/kg bw/day 2-BP in olive oil for 28 days. On the day after treatment was completed, the rats were sacrificed and the bone marrow was stained to check for micronuclei formation. 2-BP treatment did not increase the frequency of micronuclei formation. Doses were sufficient to produce toxicity in rats as suggested by decreased weight gain in all treated females and males exposed to ≥250 mg/kg bw/day. According to the authors, a dose-related reduction in polychromatic erythrocytes in all treated rats may indicate 2-BP-induced hematopoietic inhibition in the bone marrow.

Strength/Weaknesses: This paper used established in vitro methods to evaluate mutagenic potential, and in vitro and in vivo techniques to evaluate cytogenetic effects. There was a mutaginc response in two strains of Salmonella, one only with metabolic activation, one with and without metabolic activiation. The two strains both detect base-pair substitutions, raising the likelihood that 2-BP, at least under some circumstances, has mutagenic potential. The cytogenetics assays were both negative. These assays conform to accepted regulatory practices.

Utility (Adequacy) for CERHR Evaluation Process: The results of this study are directly useful in characterizing the genotoxic potential of 2-BP.

2.4 Carcinogenicity

No carcinogenicity studies were located.

2.5 Summary of General Toxicology and Biological Effects

Toxicokinetics

Evidence from occupationally exposed humans and animal toxicity studies indicate that 2-BP is absorbed following inhalation exposure. Limited information is available on the metabolism of 2-BP. Measurement of urinary metabolites in humans and rats exposed to 2-BP by inhalation suggested that 2-BP is hydrolyzed to isopropyl alcohol and bromide ion, followed by oxidation of the alcohol to acetone and excretion of bromide and acetone through urine (Kawai et al., 1997). *In vitro* experiments with rat hepatic microsomes suggested there are multiple pathways for metabolism of 2-BP (Kaneko et al. 1997). A study in rats tentatively demonstrated that 2-BP is not excreted in urine in the form of mercapturic acid or other sulfur containing conjugates (Barnsley et al., 1966).

General Toxicity

Toxicity was noted in occupationally exposed humans. Blood disorders ranging from mild anemia and leukopenia to pancytopenia with hypoplastic bone marrow were observed in male and female workers exposed to 2-BP in a Korean plant at estimated levels of 9.2-19.6 ppm with possible short term exposures of 4140.7 ppm (Kim et al., 1996a, Kim et al., 1999; Park et al., 1997). Other symptoms reported by workers included headache, dizziness, and numbness and paralysis of hand muscles. In female Chinese workers exposed to 2-BP at 0.88-16 ppm, there were significant inverse relationships between time weighted average (TWA) 2-BP exposure and red blood cell, hematocrit, and hemoglobin levels and TWA 2-BP exposure x duration of employment and hematocrit and hemoglobin levels (Ichihara et al., 1999). No relationship was found between hematological parameters and 2-BP exposure in 4 male workers exposed to 0.80-5.84 ppm. Reproductive disorders were reported in both sexes of workers in the Korean and Chines studies, these are discussed in Section 4.

An LC_{50} of 31,171 ppm was determined for mice exposed to 2-BP by inhalation for 4 hours (Kim et al., 1996b). In male rats, repeat dose inhalation toxicity studies conducted for 9-12 weeks demonstrated that 2-BP targets the hematopoietic system at concentrations ≥1509 mg/m^3 (300 ppm) and the nervous system at concentrations ≥5031 mg/m^3 (1000 ppm) (Ichihara et al., 1997; Yu et al., 1999a, 2001a). Table 2-1 illustrates the major general toxicity findings in the inhalation studies. Blood effects included reductions in erythrocytes, hemoglobin, platelets, and/or leukocytes numbers (Ichihara et al., 1997; Yu et al., 1999a, 2001a), while histopathological evaluation revealed hypocellular and fatty bone marrow (Ichihara et al., 1997). Neurotoxicity was characterized by reduced maximum nerve conduction velocity, increased distal latency, and swelling of the myelin sheath of the common peroneal nerves of the tibia (Yu et al., 1999a,

2001a). No histological effects were noted in brain or spinal cord. Nor were there clinical signs of neurotoxicity such as changes in lacrimation, salivation, pupillary response, reaction to stimuli, alertness, or pain perception, convulsions, tremors, or abnormal movements. No effects on blood chemistry parameters were observed. Reproductive effects were observed and are discussed under Section 4. Two additional studies were conducted in male rats exposed intraperitoneally. Though the route is not relevant to human exposure scenarios, the studies demonstrated adverse affects on the hematopoietic system (Yu et al., 1997) and nervous system (Zhao et al., 1999) that were qualitatively consistent to those observed in the inhalation studies.

Effects on the hematopoietic and nervous system were not determined for female rats exposed through any route. One reproductive study in female rats found decreased weight gain, activity, and muscle tonus following exposure to 5031 mg/m^3 2-BP vapors for 8 hours/day for 9 weeks (Kamijima et al., 1997). Organ weight changes included increased relative liver weight and decreased absolute spleen and absolute and relative thymus weight; no histopathological changes were noted in the organs. Reproductive effects in female rats are addressed in Section 4.

Genetic Toxicity

2-BP was mutagenic in *Salmonella* strains TA100 with metabolic activation and in TA1535 with and without activation but was negative in strains TA98 and TA1537 and in *E. coli* (Maeng et al., 1997). 2-BP did not induce chromosomal aberrations in an *in vitro* assay with Chinese hamster lung cells or micronuclei formation in Sprague Dawley rats (Maeng et al., 1997).

Carcinogenicity

No carcinogenicity data was identified.

Table 2-1. Summary of General Toxicity Inhalation Studies in Male Rats

Concentration (mg/m^3)	**Exposure Regimen**	**Sex/ Species/ Strain**	**Dose: Effect[a]**	**Reference**
1509 5031 15,092	8h/7d/9wk; whole body (9-11 d exposure period in high dose)	Male Wistar Rat	**1509 mg/m^3:** ↓ Bodyweight gain; ↓ absolute kidney weight; ↓erythrocytes and platelets. **5031 mg/m^3:** ↓ Bodyweight gain; ↓ absolute kidney and liver weight ↓erythrocytes, hemoglobin, platelets, hematocrit and leukocytes; hypocellular marrow. **15,092 mg/m^3:** ↓ Bodyweight gain; ↓erythrocytes; hypocellular marrow.	Ichihara et al. (1997)
503 5031	8h/7d/12 wk; whole body	Male Wistar Rat	**NOAEL=503 mg/m^3** **5031 mg/m^3:** ↓ bodyweight; ↓ absolute brain, liver, and kidney weight, ; ↓ erythrocytes, platelets and leukocytes; ↓ motor nerve conduction velocity and ↑distal latency; lesions in myelin sheath of tibial peroneal nerves	Yu et al. (1999a, 2001)

[a]Reproductive Effects are Summarized in Section 4.
↑=Increased Effect; ↓=Decreased Effect

3.0 DEVELOPMENTAL TOXICITY DATA

3.1 Human Data

A 26 year old woman who suffered ovarian failure after a 16 month-exposure to 2-BP in a Korean electronics plant never regained menstrual cycles but later became pregnant and gave birth to a normal full term infant (Koh et al., 1998). A six month follow-up check-up revealed that the infant was healthy. Additional details of the study are included in Section 4 (Koh et al., 1998; Kim et al., 1996a).

3.2 Experimental Animal Toxicity

Litter size was reduced in Sprague Dawley rats treated with 300 mg/kg bw and higher following 2-BP IP injection for 14 days prior to mating and during a 7-day mating period; there were no gross abnormalities observed at doses up to 900 mg/kg bw (Lim et al., 1997). Additional details of this study are included in Section 4.

3.3 Utility of Data

3.4 Summary of Developmental Toxicity

There is insufficient data upon which to evaluate the developmental toxicity of 2-BP in either humans or experimental animals. One study anecdotally reported that a healthy, full term infant was born to a 26-year-old woman who suffered ovarian failure after a 16-month exposure to 2-BP prior to the pregnancy (Koh et al., 1998). In a limited animal study, a lack of gross abnormalities was noted in the offspring of rats treated intraperitoneally with up to 900 mg/kg bw 2-BP for 2-3 weeks prior to conception (Lim et al., 1997).

4.0 REPRODUCTIVE TOXICITY

4.1 Human Data

In 1995, the National Institute of Occupational Health, Korea Industrial Safety Corporation conducted an investigation in the tactile switch assembly section of a plant where a cluster of secondary amenorrhea was reported (Kim et al., 1996a; Park et al., 1997; Kim et al. 1999). Twenty-five women and 8 men, aged 20-44 years, were employed in that part of the plant and worked 12 hours shifts. The workers were involved in a process where tactile switch parts were dipped in baths of cleaning solution located within ventilation hoods. Prior to 1994, there were 2 temporary baths without ventilation hoods. In addition to inhalation exposures, some workers were exposed dermally when they occasionally dipped their bare hands into the cleaning solution. No personal protective equipment was used. A limited number of female workers were subjected to short term exposure as they fixed problems occurring underneath the hood. Eighteen months prior to the investigation, a CFC-based cleaning solution was replaced with a solution consisting of 97.4% 2-bromopropane (2-BP) with smaller percentages of n-heptane (0.33%), 1,2-dibromopropane (0.2%), and 1,1,1-trichloroethane (0.01%). Solvent concentrations in air were not measured during actual plant operations so exposures were estimated by obtaining 14 area samples under a simulated manufacturing scenario. 2-BP levels outside the hood ranged from 9.2-19.6 ppm with a mean of 12.4 ppm. The short-term concentration of 2-BP inside the hood was measured at 4140.7 ppm and the n-heptane level was 29.8 ppm. Effects in the 2-BP exposed group were compared to a control group of 65 females and 12 males who worked in another room of the same plant. Medical histories revealed that 16 of the women exposed to 2-BPfor 4-16 months, were experiencing secondary amenorrhea. Normal menstrual cycles were reported prior to exposure. The women also had elevated follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels, but normal prolactin levels. Estradiol levels were measured in 3 women and found to be below the detection limit of 13.6 pg/ml. Progesterone withdrawal bleedings were not observed. Ten of the women complained of hot flashes. Based on symptoms, the authors diagnosed the 16 women with ovarian failure. Semen analysis revealed azoospermia in 2 men and oligospermia (<20 million sperm/ml or <50% motile) in 4 men who were exposed for a period of 16-19 months. FSH levels were around the upper normal range but testosterone levels were in the normal range. None of the men reported a loss of libido. Based on hormonal analysis, the authors concluded that germ cells and not Leydig cells were the target tissue in the affected men. Blood disorders and other systemic effects were observed in the workers and are described in detail in Section 2. The authors concluded that 2-BP was the most likely cause of health problems. Previous monitoring programs ruled out involvement by other toxic agents such as ionizing radiation, lead, formaldehyde, ethylene glycol, ether and its acetates, benzene, dinitrobenzene, and dibromochloropropane. Other confounding factors that were considered included use of oral contraceptives or any other special medications, smoking, and drinking.

Strength/Weaknesses: A strength of this study is that reproductive problems were verified by clinical measures of semen quality and ovarian ultrasound, along with serum hormones. The discussion integrates the findings and compares them with the animal toxicology data available at the time – results are consistent across species. The higher than expected incidence of reproductive effects in this cluster of cases is convincing evidence that 2-BP is likely to be a human reproductive toxicant.

These three papers report on the same cluster of cases. Park et al. (1997) is the most thorough of the three reports and it still suffers from lack of epidemiologic and laboratory rigor. None of the laboratory tests are described in sufficient detail (or even referenced) from methods standpoint. Therefore, it is not possible to judge the quality of these data. However, methodological details for tests are not typically provided in many clinical papers when the tests are considered to be standard tests. Reporting of the test

results was incomplete because the data were not presented in a table with mean values and standard deviations for measures that differed between groups. Scope of information collected by questionnaire appears to be very limited. Statistical analysis is very rudimentary with no evidence that potential confounding factors such as age (especially for peri-menopausal women), sexual abstinence (for the men), medical history or life style factors that may affect reproductive function and are associated with exposure groups were appropriately controlled for. For example, it appears that no adjustments were made to control for greater numbers of smokers in the exposure versus the comparison group. A statistical test that could have been conducted is regression analysis using several different exposure (duration of employment or exposed versus unexposed, for example) and outcome measures (hormone concentrations, presence or absence of amenorrhea, azoo/oligospermia) with adjustment for age, smoking and other potential confounders. Even more simply, bivariate analyses such as t-tests or Chi Square tests, could have been used to compare the various outcomes in the exposed and unexposed groups. Power calculations were not done but it is possible that the sample size may have been too small to make meaningful conclusions. However it is also possible that power calculations may have shown adequate power given the extremely high incidence of abnormalities. Incidence was considered by time of employment, but severity of effect was not related to exposure. It is noted that severity of effect appeared to be greatest in employees working during the period when unventilated tanks were present (February-November 1994). Table 4 of the Park et al. (1997) study shows that 17 or 18 women who started work between February and July of 1994 developed disorders, while non of those (admittedly smaller groups) who started after August 1994 developed disorders. In this regard, it would have been most useful to use November 1994 as the cut-off date. Incidence data expressed per 100 workers is misleading since the number of cases in each exposure group was very small. The "control" population appears to have been selected after the fact, which may have been problematic because the laboratory testing would then have been done at a different times than the exposed group. On the other hand, a strength of the control groups is that they were employed in the same firm, presumably in similar jobs except for the 2-BP exposure. There is no evidence that informed consent was obtained, or that the study protocol was reviewed by an IRB. There is no attempt to report participation rates and other factors indicative of potential selection bias (e.g. were the 33 workers identified the only people working in the tactile switch assembly section?).

Utility (Adequacy) for CERHR Evaluation Process: The cases in these papers are compelling, but the experimental design and analysis is questionable. Park et al., 1997 provides the most complete details of the three reports of the same cluster, but it is still a descriptive paper.

Koh et al. (1998) conducted a pathological examination and 24-month follow-up study on the 16 Korean women who suffered 2-BP-induced ovarian failure (Kim et al., 1996a). Six of the women underwent a laparoscopic examination and ovarian biopsies were conducted in 4 of those women. Results of the gross examination were varied and revealed ovaries that were either atrophied, small in size, or near normal in appearance. Biopsy results were consistent and were similar to those noted with ovarian damage from radiation or chemotherapy treatment. Findings of the biopsies included focal or diffuse fibrosis in the ovarian cortex, atrophied follicles lacking oocytes or granulosa cells, follicular developmental arrest, reduced numbers of primary follicles and corpus albicans, and hyalinization of blood vessels in the medulla. The majority of women were given estrogen-progesterone replacement therapy for 24 months. Every 6 months, the therapy was discontinued for 2-3 months to see if menstruation resumed. After twelve months of replacement therapy, consistent menstrual cycles and normal serum levels of estradiol, LH, and FSH were observed in a 24-year-old women who was exposed to 2-BP for 5 months. One 26 year old woman who was exposed to 2-BP for 16 months did not receive estrogen-progesterone replacement and did not resume menstruation. However, 7 months into the study she was found to be 6 weeks pregnant. Although serum estradiol levels were low for gestational stage, low serum LH and FSH levels suggested recovery of ovarian function. The woman delivered a normal full term infant and was able to breast feed. At 6 months of age, the infant was healthy and there were no problems with maternal lactation.

Strength/Weaknesses: This case study is a follow up on the women identified with secondary amenorhea in Kim et al. and Park et al. (above). Ovarian biopsies from six women showed fibrosis and lack of early follicles (primary and pre-antral), a histologic picture consistent with their clinical symptoms; however, in the absence of age-matched controls, conclusions of causality cannot be drawn. Hormone data shown in Table 1 of the study report is apparently the same as is shown (without statistical analysis) in Kim et al., (1996a), but follow-up hormone data is reported only for two individuals who showed spontaneous recovery of ovarian function. Again, without a second hormone assessment for the other women, lack of "recovery" of endocrine status in the other women is an assumption only.

Utility (Adequacy) for CERHR Evaluation Process: The utility of this paper is that it histologically confirms the diagnosis of ovarian failure in six of the women with amenorrhea in the Kim et al. (1996a; 1999) and Park et al. (1997) studies. The paper is not useful for risk assessment because no conclusions about the toxicity of 2-BP can be made due to a lack of similar measures in a comparison group. However, it would be unethical to subject healthy controls to an ovarian biopsy given the invasiveness of the procedure.

In 1996, Ichihara et al. (1999) conducted a study in a Chinese 2-bromopropane (2-BP) plant in order to obtain information about dose-response relationship. Personal air samples were obtained from 14 women (age 24-54 years) and 11 men (age 31-56 years) who worked 8 hours/day, 5 days/week and were employed at the plant from 5-69 months. Levels of 2-BP exceeded the detection limit of 0.2 ppm in 4 men and 12 women and ranged from 0.80-5.84 ppm and 0.88-16.18 ppm in men and women respectively. None of the personal air samples contained detectable levels of 2-BP impurities including 2-propanol (1.76%), dibromopropane (0.085%), benzene (0.055%), and trichloroethylene (0.10%). Interviews revealed amenorrhea in subjects aged 46, 47, and 54 years and polymenorrhea in 2 women aged 39 and 43 years. All of these women were employed as operators and were exposed to 2-BP at levels of 4.14-16.18 ppm. Levels of LH, FSH, and estradiol were measured and they tended to be higher in females with amenorrhea or polymenorrhea, but only the LH levels reached statistical significance when compared to female accountants and operators with normal menstrual cycles and exposure to <0.2-0.88 ppm and 4.09-8.60 ppm 2-BP, respectively. A regression analysis revealed no significant relationship between female hormonal concentrations and TWA or TWA x employment duration. A male engineer with oligoasthenozoospermia was not currently exposed to detectable 2-BP concentrations but was presumed by authors to have been exposed to high 2-BP concentrations when he set up the manufacturing process. Two males with detectable exposures to 2-BP (0.80-1.20 ppm) and 2 with non-detectable exposures had <50% sperm motility; 1 subject in each of those groups reported a shorter abstinence period compared to the other men in the study (1 vs. ≥ 3 days). Sperm from all workers met the WHO criteria for normal morphology and sperm count. Levels of LH, FSH, and testosterone were measured in men. The majority of men had LH and FSH values that were within the reference values and testosterone levels were within the reference value in all men. A regression analysis revealed no significant relationship between male hormonal concentrations or sperm indices and TWA or TWA x employment duration. Possible confounding factors such as past occupational exposures, oral contraception, medication history, nutritional status, and smoking were mentioned, but it is not clear if they were addressed. Hematological parameters were also measured in the workers and are discussed in Section 2. The authors concluded that this study did not demonstrate severe reproductive toxicity from exposure to less than 10 ppm 2-BP but noted that further studies are needed.

Strength/Weaknesses: A strength of this occupational health study was that it was conducted in a thorough manner. Informed consent was obtained, questionnaire data on work (past exposures) and medical history, reproductive history, menstrual status (women), etc. was obtained. Sufficient details on lab analyses are provided and standard World Health Organization (WHO) methods were used for semen

analysis. Exposure was monitored personally and time-weighted average exposures were calculated and used in regression analyses. Female age (>30) was controlled in the analysis.

A weakness of the study is that the number of subjects is very small, only 4 male workers were exposed to detectable 2-BP. Exposures were low (<10 ppm) since the study was done in winter; exposures are expected to be higher in the summer. There was no indication that abstinence interval was controlled/standardized in men.

Utility (Adequacy) for CERHR Evaluation Process: This study is useful since it was well done, and exposure was characterized. One Panel member suggested that the study may be useful in defining a NOEL for humans, but was not definitive due to small sample size and low exposures. However, a second Panel member suggested that the limitations of this study make it inadequate to establish a NOEL.

4.2 Experimental Animal Toxicity

4.2.1 Female Reproductive Toxicity

Kamijima et al. (1997) conducted a study in rats to clarify the effects of 2-BP-induced ovarian toxicity. Seven to nine, 15-week-old female slc: Wistar/ST rats/group (from Shizuoka Laboratory Animal Center) inhaled air, 100, 300, or 1,000 ppm (503, 1,509, 5,031 mg/m^3) 2-BP (99.4% purity) for 8 hours/day for 9 weeks. Doses were based on a previous study in male rats that demonstrated impairment of spermatogenesis at 300 ppm and serious illness at 3,000 ppm. Concentrations were monitored inside inhalation chambers. Estrous cycles were monitored for 3 weeks prior to treatment and during treatment. After the exposure ended, rats with regular estrous cycles were sacrificed on the 1st day of diestrus. Rats experiencing prolonged estrous or diestrus stages were sacrificed following treatment [the time period between the last treatment and sacrifice is not clear]. Estrous cycle data were analyzed by the Kruskal-Wallis test and body weight, organ weight, and hormonal data were analyzed by analysis of variance (ANOVA); both analyses were followed by the Dunnet-type multiple comparison method. Study results are outlined in Table 4-1. Activity, muscle tonus, and body weight gain were reduced in rats exposed to 1,000 ppm. Irregular estrous cycles were first observed around week 2 of treatment in rats of the 1000 ppm group. Five rats in the group were in continual diestrus with occasional estrous while the remaining 4 rats had continual estrous with occasional diestrus. In the 300 ppm group, a gradual prolongation of the diestrus stage was first observed at week 7 of treatment. By the end of the treatment period, all rats in the 300 ppm group had cycles consisting of continual diestrus with occasional estrus. One animal in the 100 ppm group became acyclic following 7-9 weeks of treatment, but statistical significance was not obtained. Changes in estrous cycles were accompanied by significantly reduced absolute right ovary weight in the 1,000 ppm group. A histological evaluation was conducted in ovaries fixed in 10% formalin and stained with hematoxylin–eosin. Rats experiencing persistent estrous in the 1000 ppm group had ovaries with mostly atretic follicles, very few remaining viable oocytes, thin layers of granulosa cells in cystic follicles, and no newly formed corpora lutea. The ovaries of rats in continual diestrus in the 1,000 and 300 ppm groups had reduced numbers of normal antral and growing antral follicles. Absolute and relative uterus weights were significantly decreased only in rats of the 300 and 1,000 ppm group experiencing continual diestrus. Serum LH and FSH were measured, and there were no significant differences in treated rats. Organ weight effects on non-reproductive organs were only observed in the 1000 ppm group and included significantly increased relative liver weight and decreased absolute spleen and absolute and relative thymus weight with no abnormal histological findings. The authors concluded that 2-BP was the likely cause of amenorrhea in the Korean workers exposed to 2-BP.

Strength/Weaknesses: This study and that of Yu et al., (1999b) which is apparently based on the same animals, could be criticized for using a small number of rats per group (7-9); however, variability for estrous cycle length was minimized by using only those animals with a regular 4-day cycle (according to recommendations of Cooper et al., 1993). The number of cycles in each three-week interval of treatment

was analyzed with routine statistics. Since this is actually a repeated measures design, a more appropriate statistical analysis would be that for repeated measures, using each animal as its own baseline. Hormone data did not reveal significant differences with treatment; which is surprising since LH would be expected to be high in an animal with severely damaged ovaries. This discrepancy is not adequately explained since it could indicate a second target for 2-BP, namely the brain. It would have been helpful to have estradiol and progesterone measurements in order to better interpret the meaning of the LH and FSH concentrations. Nevertheless, the study findings are convincing. The arrest of cyclicity was both time and dose-dependent. A strength is that the study was conducted for a period of time sufficient to reveal the delayed impact at the lower dose. It would be of interest to analyze paired ovarian weights since there are biological variability between ovaries (due to arbitrarily different number of large follicles or corpora lutea in each). It appears that paired ovarian weight would be significantly lower in the animals exposed to 1,000 ppm (Table 2 of the study). Significant effects on estrous cyclicity occurred at a dose (300 ppm) lower than that producing significant effects on body weight (1000 ppm). A weakness was that ovarian histology was reported only in a qualitative manner; however, the Yu et al. (1999b) paper dealt with quantification of variously staged follicles, so that, together, the papers provide strong evidence for ovarian toxicity to primordial and small follicles.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate for use in risk assessment. Data show clear dose- and time-dependent effects on estrous cyclicity which provides evidence of adverse effects on reproductive organ (ovarian) function, even in the absence of fertility data. Lack of effect on serum LH/FSH is inconsistent with the cyclicity effect, however.

Table 4-1. Major Effects in Wistar Rats in Reproductive Toxicity Study by Kamijima et al. (1997).

Number	Dose (ppm)	Effects
7	0	
8	100	No effects.
7	300	↑ Irregular estrous cycles. ↓ Absolute and relative uterus weight. ↑ Ovarian histopathology.
9	1000	↑ Irregular estrous cycles. ↓ Absolute right ovary weight and absolute and relative uterus weight. ↑ Ovarian histopathology. ↓ Bodyweight gain. ↓ Activity and muscle tonus. ↑ Relative liver weight. ↓Absolute spleen and absolute and relative thymus weight.
Protocol: Female rats exposed to 2-BP vapors for 8 hours/day for 9 weeks. **Notes:** ↑,↓=Statistically significant increase, decrease.		

Yu et al. (1999b) conducted a dose-response and a time-course experiment to identify the target cell and define the mechanism of toxicity for 2-BP-induced ovarian toxicity. Both studies used female Wistar rats (from Shizuoka Laboratory Animal center) that were 12-weeks-old at the start of dosing and monitored estrous cycles for 3 weeks prior to and during treatment. Animals were exposed in chambers to air or 2-BP (99.5%) and chamber concentrations were monitored. Doses were based on a previous study in male rats that demonstrated impairment of spermatogenesis at 300 ppm and serious illness at 3,000 ppm. At sacrifice, the right ovary was fixed in 10% neutral buffered formalin, stained with hematoxylin–eosin, and

examined histologically. Follicular counts were analyzed by ANOVA followed by the Tukey-Kramer multiple comparison test. Results are outlined in Table 4-2. In the dose-response experiment, 7-9 rats/group were exposed to 2-BP vapors at 0, 100, 300, or 1,000 ppm [503, 1,509, 5,031 mg/m^3] for 8 hours/day for 9 weeks. Following the exposure period, the rats were sacrificed on the day of diestrus I. Estrous cycles were disrupted after 7 weeks of treatment in the 300 and 1000 ppm groups, while changes in the ovary were seen at all dose levels. The numbers of primordial and growing follicles were significantly reduced in all dose groups (≥100 ppm) and numbers of antral follicles were significantly reduced at the two highest doses (300 and 1,000 ppm). Ovaries of rats from the mid and high dose groups were hypoplastic and contained few or no corpora lutea. For the time course-experiment, rats were exposed to 0 or 3,000 ppm 2-BP vapors for 8 hours/day and then sacrificed at 1, 3, 5, or 17 days following exposure. Seven rats/group were sacrificed at each time period while in estrous. Commencement of exposure was timed according to the rat's cycle to ensure that rats would be in estrous at the time of sacrifice. The right ovary was examined histologically as described above for the dose-response experiment. The left ovary was examined for apoptotic cells by labeling DNA strand breaks through incorporation of digoxigenin-conjugated deoxyuridine 5'-triphosphate (d-dUTP). In the time-course experiment, there was no effect on estrous cycles. Ovarian histopathology consisted of oocytes with distorted symmetry and nuclei on day 5 and pyknotic cells and oocyte nuclei shrinkage on day 17. Numbers of primordial follicles began decreasing on day 5 and reached statistical significance on day 17. Apoptosis was noted in oocytes and granulosa cells of primordial follicles after 5 days of exposure. The authors concluded that 2-BP induced ovarian toxicity through the destruction of primordial follicles and oocytes by apoptotic processes. They postulated that estrous cycles were subsequently disrupted when recruitment of growing and antral oocytes could no longer be supported. Therefore, follicle counts were more sensitive than monitoring of estrous cycles for detecting 2-BP-induced ovarian toxicity.

Strength/Weaknesses: The first experiment described in this study report appears to use the same animals as that of Kamijima et al. (1997) to evaluate folliculogenesis in a quantitative manner so as to identify the ovarian target(s). Differential follicle counts were made according to previously published, and widely accepted methods. Significant decreases in primordial, growing and antral follicles seen at the higher doses are consistent with irregular cycles and acyclicity in the Kamijima et al., (1997) study. However, differential follicle counts showed that 100 ppm was also an effective dose. The time-course study adds information about targets since it shows that primordial follicles are the first to be affected by a single (one day) high dose. This suggests that decreases in other follicle populations are mainly the result of maturation depletion.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate for use in risk assessment. Differential follicle counts establish a LOAEL of 100 ppm which is below that determined for estrous cyclicity. The time response study provides additional evidence that 2-BP is targeting primoridal follicles selectively, and provides evidence that these follicles and/or the oocytes within them die by apoptosis.

Table 4-2. Major Effects in Reproductive Toxicity Study in Wistar Rats by Yu et al. (1999b).

Number	Dose (ppm)	Effects
7	0[a]	
7	100[a]	↓ Primordial follicles (55% of control). ↓ Growing follicles (55% of control).
8	300[a]	↑ Irregular estrous cycles. ↓ Primordial follicles (55% of control). ↓ Growing follicles (50% of control). ↓ Antral follicles (50% of control).
9	1000[a]	↑ Irregular estrous cycles. ↓ Primordial follicles (20% of control). ↓ Growing follicles (25% of control). ↓ Antral follicles (20% of control).
7	0[b]	
7/time point	3000[b]	↑ Oocyte distortions (day 5). ↑ Pyknotic cells and oocyte nuclei shrinkage (day 17). ↓ Primordial follicles (day 5). ↑ Apoptosis in primordial follicles (day 5). No effect on estrous cycle.
Protocol: [a]Twelve-week-old female Wistar rats exposed to 2-BP vapors for 8 hours/day for 9 weeks. [b]Female rats exposed to 2-BP vapors 8 hours/day and then sacrificed at 1, 3, 5, or 17 days following exposure. **Notes:** ↑,↓=Statistically significant increase, decrease.		

Lim et al. (1997) examined 2-BP toxicity in rats to clarify effects on female reproductive function. Ten, 8-week-old female Sprague-Dawley (Crj:CD) rats/group (from Daehan Animal Center) were injected i.p. with 2-BP (99.0%) in olive oil at 0, 300, 600, or 900 mg/kg bw for 14 days prior to mating and during a 7 day mating period to untreated rats at a ratio of one male to each female. [The rational for dose selection was not discussed.] Estrous cycles were monitored 2 weeks prior to and during treatment. Dams that had pups were sacrificed one day after giving birth and dams with no pups were sacrificed 28 days after mating. [The evaluation of dams did not include counts of corpora lutea and implantation sites]. Body weight data were analyzed by two-way ANOVA and Duncan's multiple range test; fertility data were analyzed by chi-square test. Results are outlined in Table 4-3. Maternal weight gain was reduced in all treated groups and terminal body weights were significantly lower in the 600 and 900 mg/kg bw group. However, there were no corrections made for gravid uterine weight. One dam in the 600 mg/kg group died due to internal bleeding from the perforation of a blood vessel during injection. One dam in the 300 mg/kg bw group and 3 dams in the 900 mg/kg bw group died of unknown causes during the post-treatment period. Narcosis was reported for rats in the 600 and 900 mg/kg groups. The length of the estrous cycle was increased in the 900 mg/kg bw group due to prolongation of the diestrus stage. Relative ovary weights were significantly reduced in the high dose rats but there were no effects on relative kidney, spleen, or liver weight. Histological evaluations were not conducted. Effects on reproductive

function were noted but the statistical significance was not discussed. As discussed in greater detail in Table 4-3, treatment with 2-BP resulted in dose related decreases in fertility, number of dams giving birth, and number of pups born, but there were no abnormal pups observed. Gestation length was unaffected by 2-BP treatment. Effects on pup body weight could not be determined. The authors concluded that their study indicated 2-BP as the causative agent of amenorrhea in female workers exposed to 2-BP, but noted that additional studies including measurements of gonadotropin levels are needed.

Strength/Weaknesses: No rationale for the dosages used was provided, nor were calculations made to permit comparisons with doses in inhalation studies. These dosages were apparently quite high as narcosis was evident in the two higher exposure groups (600 and 900 mg/kg). Importantly, narcosis can indicate sufficient neurotoxicity as to impair the LH surge and this could be another mechanism by which estrous cyclicity is impaired. It is hard to attribute decreased weight gain (Fig. 2 in study) to the exposure since it could be due to lack of pregnancy. Duration of exposure was short considering that significant effects in inhalation studies described above were not seen until 5–7 weeks of exposure. However, a non-significant disruption in estrous cyclicity has been noted following exposure to 1,000 ppm 2-BP for 1-3 weeks (Kamijima et al. 1997). Lack of effect on estrous cyclicity at 300 and 600 mg/kg could be due to short exposure period. Irregular cycles seen only in high dose group don't explain the infertility seen in some rats at 300 and 600 mg/kg. Text says Chi square was used to evaluate reproductive effects related to fertility indices, but Table 4 in the study does not indicate where statistically significant differences were found.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited utility since route of exposure is not appropriate, dosages appear to be quite high, and duration of exposure was relatively short. Effects at high dose on estrous cyclicity are at least consistent with observations made in inhalation studies.

Table 4-3. Major Effects in Reproductive Toxicity Study in Sprague-Dawley Rats by Lim et al. (1997).

Number[a]	Dose (mg/kg bw)	Effects
10/10	0	
10/9	300	↓ Weight gain. Death in 1 dam. ↓ Fertility (78 vs. 90%)[b]. ↓ Dams with live pups (n=6 vs 9)[b]. ↓ Litter size (7.3 vs 9.8)[b].
10/9	600	↑ Narcosis. ↓ Weight gain. ↓ Terminal body weight (uncorrected). ↓ Fertility (33 vs. 90%)[b]. ↓ Dams with live pups (n=3 vs 9)[b]. ↓ Litter size (6.7 vs 9.8)[b].
10/9	900	↑ Narcosis. ↓ Weight gain. ↓ Terminal body weight (uncorrected). Death in 3 dams. ↓ Relative ovary weight. ↑ Estrous cycle length from 4.6 days to 11.05 days prior to and after treatment. ↓ Fertility (11 vs. 90%)[b]. ↓ Dams with live pups (n=1 vs 9)[b]. ↓ Litter size (1 vs 9.8)[b]. No effect on gestation length and no pup abnormalities at any dose.
Protocol: Female, 8-week-old rats were injected IP with 2-BP from 14 days prior to mating and during a 7-day mating period. **Notes:** ↑,↓ Statistically significant increase, decrease. [a]Number of rats at beginning of study/number of rats copulating with untreated males. [b]Statistical significance is not known.		

Sekiguchi and Honma (1998) conducted a study to determine the effects of 2-BP on ovulation. Four to five, 51-53 day old female ICR mice/group (from Charles River, Japan) were injected i.p. with 2-BP [purity not reported] in olive oil at 500, 1,000, or 2,000 mg/kg. [Rational for dose selection was not reported]. Mice were given 8 injections every 2-3 days over a period of 17 days. Ovulation was induced by i.p. injection with pregnant mare's serum gonadotropin and human chorionic gonadotropin on the 15th and 17th day of 2-BP treatment. Mice were sacrificed and necropsied the day after the last treatment and liver, uterus and oviduct were examined. Data were analyzed by Dunnett's multiple comparison. Results are outlined in Table 4-4. Body weight gain was reduced in the 1,000 and 2,000 mg/kg bw groups with statistical significance achieved at 2,000 mg/kg bw. However terminal body weights did not differ from controls. One mouse in the 2,000 mg/kg bw group died. The number of ovulated ova was significantly reduced in the 1,000 and 2,000 mg/kg bw groups. A non-significant reduction in absolute and relative

uterus weight was also observed in the 2,000 mg/kg group. There was no mention of histopathological evaluation. The authors concluded that results were consistent with humans experiencing 2-BP intoxication. It is noted that this study was published as a short communication.

Strength/Weaknesses: The rationale for using mice was not provided. The assay evaluates the ability of the ovary to respond to gonadotropins and is an indirect measure of the number of competent or recruitable follicles in the ovary. Results indicate that high levels of 2-BP (1000 or 2000 mg/kg) given in 8 ip injections over 17 days, significantly reduce the number of oocyte ovulated after induction of superovulation. Other symptoms (e.g. narcosis) are not mentioned, so it is difficult to attribute effects to ovarian toxicity per se. The authors overinterpret their data, especially in relating it to the epidemiology data.

Utility (Adequacy) for CERHR Evaluation Process: There is not sufficient data for this study to be useful for risk assessment. The inappropriate exposure route and systemically toxic doses also make this study of limited utility for risk assessment. The study provides **indirect** evidence for depletion of follicle pools in mice, as has been reported in rats.

Table 4-4. Reproductive Toxicity Study in ICR mice by Sekiguchi and Honma (1998).

Number	Dose (mg/kg bw)	Effects
5	0	
5	500	No effects.
5	1000	↓ Number of ova ovulated (23.8 vs 52.3).
4	2000	↓ Number of ova ovulated (6.0 vs 52.3). ↓ Bodyweight gain.
Protocol: 51-53-day old female ICR mice were I.P. injected with 2-BP a total of 8 times over 17 days. **Notes:** ↑,↓=Statistically significant increase, decrease.		

4.2.2 Male Reproductive Toxicity

Ichihara et al. (1997) conducted a study to determine the testicular and hematopoietic toxicity of 2- BP in 13-week-old Wistar rats (from Shizuoka Laboratory Animal Center). Reproductive parameters are addressed in this section while hematological and other systemic effects are outlined in Table 4-5 and discussed in detail in Section 2. Nine male rats/group were exposed by inhalation to air or 300, 1,000, or 3,000 ppm [1,509, 5,031 or 15,092 mg/m^3] 2-BP (99.4% purity) for 8 hours/day, 7 days/week. The maximum concentration was about 10% of the LC_{50}. Concentrations were monitored inside inhalation chambers. The controls and 2 lowest dose groups were exposed for 9 weeks. However, exposure in the high dose group, ended after 9-11 days due to excessive toxicity. Three rats in the high dose group were sacrificed immediately after exposure and their testes and femur were examined histologically. The remaining 6 rats were exposed to filtered air for the remainder of the exposure period and evaluated for all parameters in this study. Reproductive organs were weighed at sacrifice. The left testis and epididymis were preserved in Bouin's solution and the prostate and seminal vesicle in 10% neutral buffered formalin. Testis and epididymis were stained with periodic acid-Schiff's reagent and other tissue sections with hematoxylin-eosin. Abnormal sperm data were analyzed by Student's t-test and all other data by ANOVA followed by Dunnett's multiple comparison method. Significant, dose-related effects first noted at the low dose (300 ppm) included reductions in absolute and relative epididymides and testes weight,

and absolute prostate, and seminal vesicles weight. Significant dose-related sperm effects noted at 300 ppm and higher included reduced counts and motility and increases in tailless sperm. An increase in abnormal sperm (hooked or reflexed head) was noted at 300 ppm but could not be evaluated at higher doses because there were very few intact sperm remaining. Testicular lesions were observed at all dose levels and included atrophy of seminiferous tubules, reductions in germ cells, and hyperplastic Leydig cells that increased in severity at higher doses. Vacuolation of Sertoli cells was also observed in 2 of the 3 rats sacrificed immediately after exposure to 3,000 ppm for 9-11 days. Slight atrophic changes were reported for seminal vesicles and prostate of treated rats.

Strength/Weaknesses: Although there was a small number of male rats per group (9), multiple outcomes of reproductive function were obtained, including good quality histology, and sperm measures (counts, morphology, motility). Treatment was of sufficient duration (9 weeks) to detect effects on all stages of spermatogenesis. Dose range included a toxic level (3000 ppm), but not a no-effect level. Serum hormones were not measured, but these are of limited value at the end of a subchronic exposure interval anyway. Study would have been more informative if interim observations had been made. Ceasation of dosing after 9-11 days in high dose group resulted in demonstration that severe testicular toxicity apparent at high dose does not appear to be reversible, at least in the short term.

Utility (Adequacy) for CERHR Evaluation Process: The study is useful for risk assessment in that it provides convincing evidence for testicular toxicity of 2-BP in a dose responsive manner. However, it does not identify a NOAEL. The study indicates lack of reversibility at the high dose within the time frame and conditions of this study. It also shows that effect appears specific for blood and testes (vs. liver or kidneys), and that testes might be more sensitive than blood. Results are consistent with effect on Sertoli cells (alterations in sperm morphology and motility), and on spermatogonia (depletion of sperm numbers). Effects on accessory organs are indicative of low testosterone, although serum hormones were not measured. This could be secondary to direct effects on testis, but the design does not rule out more direct endocrine effects.

Table 4-5. Reproductive Toxicity Study in Wistar Rats by Ichihara et al. (1997)

Number	Dose (ppm)	Effects
9	0	
9	300	↓ Absolute epididymides, testes, prostate, seminal vesicles, and kidney weight. ↓ Relative epididymides and testes weight. ↓ Sperm count (358 vs. 569x10^6/g cauda). ↓ Sperm motility (16 vs 86%). ↑ Tailless sperm (56 vs. 10%). ↑ Abnormal sperm (21 vs. 7%). ↑ Testicular lesions. ↓ Erythrocytes and platelets. ↓ Body weight gain.
9	1000	↓ Absolute epididymides, testes, prostate, seminal vesicles, liver and kidney weight. ↓ Relative epididymides, testes, prostate and seminal vesicles weight. ↓ Sperm count (326 vs. 569x10^6/g cauda). ↓ Sperm motility (0 vs 86%). ↑ Tailless sperm (98 vs. 10%). ↑ Testicular lesions. ↓ Erythrocytes, hemoglobin, hematocrit, platelets, and leukocytes. ↑ Bone marrow lesions. ↓ Body weight gain.
6	3000	↓ Absolute epididymides, testes, prostate, and seminal vesicles weight. ↓ Relative epididymides and testes weight. ↓ Sperm count (151 vs. 569x10^6/g cauda). ↓ Sperm motility (0 vs 86%). ↑ Tailless sperm (99 vs. 10%). ↑ Testicular lesions. ↓ Erythrocytes. ↑ Bone marrow lesions. ↓ Body weight gain.
Protocol: 13-Week-old male Wistar rats inhaled 2-BP vapors for 8 hours/day, 7 days/week for nine weeks in 2 lowest dose groups and 9–11 days in highest dose group. **Notes:** ↑,↓=Statistically significant increase, decrease.		

Yu et al. (2001a) noted atrophy of seminiferous tubules and loss of germ cells in the testes of 10-week-old Wistar rats exposed to 1,000 ppm [5,031 mg/m^3] 2-BP vapors for 8 hours/day, 7 days/week, for 12 weeks; no lesions were observed following exposure to 100 ppm 2-BP. Additional details of this study are included in Section 2.

Strength/Weaknesses: This neurotoxicology study provides confirming evidence that 1000 ppm causes testicular atrophy after subchronic (10 wks) exposure by inhalation, and adds histologic evidence that 100 ppm may be a NOAEL for testicular toxicity. Appropriate fixation appears to have been conducted for testicular evaluation, so the observations, though limited in scope, appear reliable.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate to suggest that 100 ppm is a no effect level after subchronic inhalation exposure, and confirm testicular toxicity at 1000 ppm (in presence of changes in hematological indices and peripheral neuropathology). The study also provides evidence that 1-BP did not cause the same hematological changes or testicular toxicity at 1000 ppm (5-7 weeks), but was a more potent neurotoxicant than 2-BP.

Yu et al. (1997) conducted a 2-BP toxicity study in rats to verify that adverse effects in the hematopoietic and reproductive systems of workers of a Korean electronics plant were due to 2-BP exposure. Ten male Sprague-Dawley rats/group (~12 weeks old; purchased from Daehan Animal Center) were injected intraperitoneally (IP) with 0, 125, 250, or 500 mg/kg bw 2-BP (99% purity) in olive oil, 6 times/week for 4 weeks. The authors acknowledged that the administration route does not pertain to occupational exposures, but stated that inhalation tests are required only if negative results are obtained with IP exposure. [The rational for dose selection was not discussed.] This summary describes the reproductive effects while non-reproductive findings are discussed in Section 2. Results of the study are outlined in Table 4-6. Rats exposed to 2-BP at 250 and 500 mg/kg bw experienced a significant, dose-related reduction in relative testicular weight. Histological examination of testes (preserved in 10% formalin and stained with hematoxylin–eosin) revealed severely atrophic tubules with germ cell necrosis of spermatogonia and spermatocytes, vacuolized Sertoli cells, and hyperplasia and hypertrophy in Leydig cells in the 2 highest dose groups (250 and 500 mg/kg bw). Epididymal atrophy with vacuolization of the epithelium was also observed, but the dose at which this effect first occurred was not specified.

Strength/Weaknesses: This neurotoxicity study of 1-BP and 2-BP, included reproductive outcomes. Results are limited by short exposure duration (28 days) which can miss effects on spermatogonia. However, severity of effect allowed its detection. The route of exposure (ip) is not relevant for humans.

Utility (Adequacy) for CERHR Evaluation Process: This study could be useful for route-to-route extrapolation if ip route were used for mechanistic studies in the future. But dose response data suggests that 125 mg/kg ip is the NOAEL for testicular toxicity, although other toxicities are seen at this dosage. The study also shows that testicular atrophy can be produced with shorter exposures (28 days vs. 9 - 10 weeks) at high dose.

Table 4-6. Major Effects in Reproductive Toxicity Study in Sprague-Dawley Rats by Yu et al. (1997).

Number	Dose (mg/kg bw)	Effects
10	0	
10	125	Clinical signs.
10	250	Clinical signs. ↓ Bodyweight gain. ↓ Relative testes weight. ↑ Seminiferous tube atrophy with, germ cell necrosis, Sertoli cell vacuolization, and Leydig cell hyperplasia. ↑ Relative adrenal weight.
10	500	Clinical signs. ↓ Bodyweight gain. ↓ Relative testes weight. ↑ Seminiferous tube atrophy with, germ cell necrosis, Sertoli cell vacuolization, and Leydig cell hyperplasia. ↑ Relative adrenal, lung, spleen, liver and brain weight. ↓ White blood cell, lymphocyte, platelets, and hemoglobin. ↓ Blood alkaline phosphates activity. ↑ Cholesterol.
Protocol: 12-week-old Sprague-Dawley rats injected i.p. with 2-BP on 6 days/week for 4 weeks. **Notes:** ↑,↓ Statistically significant increase, decrease.		

Wu et al. (1999a) conducted a reproductive toxicity study to obtain information about 2-BP toxicity in mature (9-week-old) and immature (5-week-old) male Sprague Dawley rats (Sino-British SIPPR/BK Animal Co.). Six rats/dose/age group were injected subcutaneously (s.c.) with 0, 200, 600, or 1,800 mg/kg bw 2-BP (99.6% purity) on 5 days/week with treatment lasting for 5 and 7 weeks in mature and immature rats respectively. [There was no mention of vehicle]. Authors stated that although exposure through the s.c. route does not occur in occupational settings, s.c. injection was chosen to assure complete and rapid absorption. The basis for dose selection was previous data and preliminary results. After treatment, reproductive performance was assessed in the mature rats by mating them 1:2 with untreated females over 7 days. Sperm quality and testicular histology (fixed in 10% formalin and stained with hematoxylin–eosin) were examined in both age groups. Mature rats were sacrificed 4 days after mating to allow for restoration of sperm levels. Immature rats were sacrificed immediately after the treatment period. Analysis of data included ANOVA and Dunnett's test for weight effects, the Kruskal-Wallis or Mann-Whitney test for sperm, fetal, and hormonal data, and the chi-square test for reproductive function data. Statistically significant results are listed in Table 4-7. Several effects were noted in mature and immature rats at the lowest dose (200 mg/kg bw) and included dose-related reductions in sperm count and viability, and increases in deformed sperm and testicular lesions. Testicular lesions increased in severity according to dose and included atrophied seminiferous tubules with reductions in germ cell numbers. Serum testosterone levels were first reduced at 200 and 600 mg/kg bw in mature and immature rats, respectively. A dose related reduction in absolute and relative testicular weight was first noted in mature and immature rats at the 600 mg/kg bw dose. Additional effects seen at the highest dose level (1,800 mg/kg bw) in both age groups included reductions in absolute epididymis, prostate seminal vesicle, and pituitary weight and relative epididymis weight. Dose-related adverse effects on the reproductive

performance of mature rats were first noted at 600 mg/kg bw and included reduced mating and pregnancies and an increase in the number of days for pregnancy initiation to occur. Effects also noted in dams mated with the high dose group (1,800 mg/kg bw/day) included a reduced number of implantation sites and increased fetal mortality. Expression of β-luteinizing hormone was measured in mature rats and was found to be increased in rats treated with 1,800 mg/kg bw. Based on testicular and sperm effects, the authors estimated that the NOAEL for 2-BP was below 200 mg/kg bw/day.

Strength/Weaknesses: Strengths of this study include assessment of sperm counts, viability and morphology, as well as serum testosterone levels The small number of rats per group (6) limits the power to detect effects. The design does not really evaluate immature rats. Use of longer dosing time (7 weeks) in young rats (5 weeks of age) means that they were adult (84 days old) when evaluated (vs. 98 days old for "adult" group). Therefore, they were exposed during adolescence and adulthood, and one would expect effects to be the same in both groups. The subcutaneous route is not directly relevant for human inhalation exposures. Another weakness is that the testosterone assay is not adequately described, and fixation of testis for histology is not optimal. Effects at two higher dosages were accompanied by severe weight loss, so other effects are questionable as to their specificity. Fertility indices should be calculated and analyzed with the male as the unit of measure since only males were treated (see table 5 of study).

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited value due to the subcutaneous route of exposure. Testicular toxicity at high dosages is of questionable specificity since body weight was down more than 10%. The study demonstrates less severe effects at lower doses that do not affect weight, therefore it confirms inhalation studies that demonstrate that 2-BP is a testicular toxicant. Hormone (or LH mRNA) changes are probably secondary to testicular and general toxicity at high dose and not a primary effect.

Table 4-7. Major Effects in Reproductive Toxicity Study in Sprague-Dawley Rats by Wu et al. (1999a).

Number/ Age Group	Dose (mg/kg bw/day)	Effects in Mature Rats	Effects in Immature Rats
6	0		
6	200	↓ Sperm count (61.6 vs 140.6x10^6/ml) and viability (52.3 vs 70.8%). ↑ Deformed sperm (9.6 vs 2.1%). ↓ Serum testosterone (97.5 vs 272 fmol/ml). ↑ Testicular lesions.	↓ Bodyweight gain. ↓ Sperm count (61.5 vs 74.6x10^6/ml) and viability (51.6 vs 64.2%). ↑ Deformed sperm (8.5 vs 4.2%). ↑ Testicular lesions.
6	600	↓ Bodyweight gain. ↓ Absolute and relative testis weight. ↓ Sperm count (54.6 vs 140.6x10^6/ml) and viability (25.4 vs 70.8%). ↑ Deformed sperm (12.6 vs 2.1%). ↓ Serum testosterone (82.2 vs 272 fmol/ml). ↑ Testicular lesions. ↓ Mating (75 vs100%). ↓ Pregnancies (78 vs 100%). ↑ Days to fertilization (3.9 vs 2.8).	↓ Bodyweight gain. ↓ Absolute and relative testis weight. ↓ Sperm count (38.0 vs 74.6x10^6/ml) and viability (40.7 vs 64.2%). ↑ Deformed sperm (10.8 vs 4.2%). ↓ Serum testosterone (131 vs 153 fmol/ml). ↑ Testicular lesions.
6	1800	↓ Bodyweight gain. ↓ Absolute testis, epididymis, prostate, seminal vesicle, and pituitary weight. ↓ Relative testis and epididymis weight. ↓ Sperm count (11.3 vs 140.6x10^6/ml) and viability (0 vs 70.8%). ↑ Deformed sperm (75.2 vs 2.1%). ↓ Serum testosterone (80.8 vs 272 fmol/ml). ↑ Testicular lesions. ↓ Mating (42 vs 100 %). ↓ Pregnancies (20 vs 100%). ↑ Days to fertilization (4.6 vs 2.8). ↓ Implantations/litter (7.4 vs 11.2). ↓ Viable fetuses/litter (6.0 vs 10.2). ↑ Resorptions (5.8 vs 1.6%). ↑ β-LH gene expression in pituitary.	↓ Bodyweight gain. ↓ Absolute testis, epididymis, prostate, seminal vesicle, and pituitary weight. ↓ Relative testis and epididymis weight. ↓ Sperm count (7.8 vs 74.6x10^6/ml) and viability (0 vs 64.2%). ↑ Deformed sperm (93.6 vs 4.2%). ↓ Serum testosterone (129 vs 153 fmol/ml). ↑ Testicular lesions.
Protocol: Mature (9 week-old) and immature (5-week-old) rats were s.c. injected 5 days/week with 2-BP for 5 and 7 weeks, respectively. Mature rats were mated 1:2 with untreated females. **Notes:** ↑,↓=Statistically significant increase, decrease.			

Omura et al. (1997a, 1997b; 1999) used quantitative histopathology to examine mechanisms of 2-BP-induced testicular toxicity. They counted the numbers of different types of germs cells in seminiferous tubule cross sections selected to represent specific stages of spermatogenesis (Creasy, 1997). While this level of quantification is not required by OECD or EPA test guidelines, it does allow detection of subtle

changes in the testis. Such information may provide insights into cellular targets and mechanism of action of a toxicant, especially after short term exposure (Creasy, 1997), and can be used in risk assessment (U.S. Environmental Protection Agency, 1996).

Omura et al. (1997a; 1997b) utilized this approach to determine the type(s) of testicular cells targeted by 2-BP exposure. Four, 11 week-old Kud: Wistar rats/group were injected s.c. with saline or 1,355 mg/kg 2-BP (>99% purity) for 5 days/week for 2 weeks. This exposure duration was selected to cover one spermatogenic cycle. According to the study authors, that dosage is equivalent to inhalation of 1,000 ppm for 8 hours at a respiratory minute volume of 215 ml/min/383 g bw. Following treatment, reproductive organs were weighed, testicular and epididymal sperm were counted, and sperm motility and morphology were assessed. Abnormal sperm data was evaluated by the Mann-Whitney test and other data by Student's t-test. Body weight gain was significantly reduced by 2-BP treatment (by 13%). The treated group also had significantly reduced absolute seminal vesicle weight and increased relative epididymides weight. Sperm count per testis was significantly reduced in treated rats, but 2-BP treatment had no significant effect on sperm count per gram testis weight, motility, or abnormalities. Histological evaluation of the right testis (preserved in Bouin's and stained with periodic acid schiff reagent) revealed mild atrophy in only a few seminiferous tubules. The number of germ cells and Sertoli cells in seminiferous tubules at stage I, V, VII, X, and XII were determined. Treatment with 2-BP significantly reduced the numbers of spermatogonia and stage specific spermatocytes , without affecting later cell types (spermatids) (see Table 4-8). Based on the changes in germ cell numbers during each stage of the cycle evaluated, the authors estimated that spermatogonia were the target of 2-BP exposure. Because spermatogonia develop into spermatocytes, the authors believed the reductions in spermatocytes to be due to depletion of spermatogonia. However, they stated that further studies are needed to confirm the effects of 2-BP toxicity.

Strength/Weaknesses: Methods for fixation, histologic processing, staining and staging of seminiferous tubules were appropriate. Timing of dosing and sacrifice are appropriate for distinguishing between spermatogonial and spermatocyte toxicity.

Utility (Adequacy) for CERHR Evaluation Process: This study is useful for identifying the target cell (spermatogonia) following an acute exposure to a high dose of 2-BP at a dose equivalent to that causing testicular atrophy in subchronic exposure studies.

Table 4-8. Effect on germ cells numbers in Omura et al. (1997a) study.

Stage	Effect on Germ Cell Numbers (% of control values)
VII	↓ Spermatogonia (45%)
	↓ Preleptotene spermatocytes (5%)
X	↓ Spermatogonia (35%)
	↓ Leptotene spermatocytes (5%)
XII	↓ Spermatogonia (65%)
	↓ Zygotene spermatocytes (5%)
I	↓ Spermatogonia (20%)
	↓ Pachytene spermatocytes (70%)
V	↓ Spermatogonia (5%)
	↓ Pachytene spermatocytes (50%)

Omura et al. (1999) conducted a second study to confirm that spermatogonia are the target cells of 2-BP exposure. Eleven-week-old Kud: Wistar rats were injected s.c. with 1,355 mg/kg bw 2-BP (>99% purity) without vehicle for 1-5 days. Groups of 4 rats were sacrificed 6 hours after treatment on day 1, 2, 3, 4, and 5. Five control rats were killed after 5 days of saline injection. Enumeration of spermatogenic cell types was conducted in a stage-specific manner as described above in the Omura et al. (1997a) study. Spermatogenic cell number data were analyzed by one-way ANOVA followed by Fisher's least significant difference procedure. As noted in Table 4-9, 2-BP exposure significantly reduced spermatogonia numbers in Stages XII, I, II-III, and V. In stage I, spermatogonia numbers were reduced on each day with greater reductions occurring with increased time of treatment. A delay in the division of type B spermatogonia was also observed in rats treated for 5 days. In contrast to the Omura et al. (1997a) study with a 2-week exposure period, spermatocytes were generally unaffected . Statistically significant but slight reductions in pachytene spermatocyte numbers (90-95% of control values) were only observed in Stage I, but the numbers did not decrease with increased time of treatment. The authors stated that the reduction in pachytene spermatocytes had no biological significance and may have resulted from high numbers in control rats. The authors concluded that the early reduction in spermatogonia numbers demonstrated that spermatogonia are the target cells of 2-BP exposure.

Strength/Weaknesses: This study was done according to proper methods for sample preparation and evaluation (as with their 1997 study). Shorter exposure periods help refine evaluation of sensitive cell types and increase confidence that effect was confined to the spermatogonia. The dosage used was high and probably, but not necessarily, reflective of the primary target at lower doses.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate for defining the cellular target of 2-BP, and results are consistent with the spectrum of effects seen after subchronic exposure. Knowledge about target cells is useful in comparing mechanisms of 1-BP and 2-BP and can be used in risk assessment.

Table 4-9. Time-dependent reductions in Spermatogonia numbers observed by Omura et al. (1999).

Stage VII	Effect on Spermatogonia numbers on each day of treatment (% of control values)				
	Day 1	Day 2	Day 3	Day 4	Day 5
	-	-	-	-	-
X	-	-	-	-	-
XII	-	-	↓ (80%)	-	↓ (70%)
I	↓ (70%)	↓ (90%)	↓ (60%)	↓ (50%)	↓ (15%)
II-III	-	↓ (65%)	-	↓ (70%)	↓ (25%)
V	-	-	-	-	↓ (60%)

To characterize testicular toxicity resulting from 2-BP exposure in rats, Son et al. (1999) compared histological observations of spermatogenic staging to quantitative spermatogenesis measurements. Ten-week-old Sprague-Dawley rats (from Screening and Toxicology Research Center) were gavaged with 0 or 3,500 mg/kg bw/day 2-BP [purity not reported] in corn oil for 3 days. Three control and 5 treated rats/day were sacrificed at 1, 3, 5, 7, 14, 28, 42, or 70 days following treatment. Histological examination of testes fixed in Bouin's solution and stained with hematoxylin–eosin or periodic acid Schiff's reagent revealed that the sequence of toxicity was damage to spermatogonia, leading to depletion of spermatocytes, spermatids, and spermatozoa that ultimately resulted in testicular atrophy (Table 4-10). Leydig cell hyperplasia was also observed on the last observation day. Immunohistochemical staining of Leydig cells with proliferating cell nuclear antigen (PCNA), a marker of cell proliferation, confirmed the

histological observation. Evidence of partial recovery was noted by some regeneration of germ cells and spermatocytes towards the end of the observation period. Electron microscopic examination of testes from 1 rat/group/sacrifice day confirmed the histological effects. Flow cytometry was used to quantify spermatogenesis based on DNA ploidy of testicular cells. The technique measured the percentages of haploid (spermatozoa, spermatids), diploid (spermatogonia, secondary spermatocytes, and Sertoli , Leydig , and connective tissue cells) and tetraploid (primary spermatocytes) cells. Data were analyzed by Dunnett's test. Results were consistent with histological findings as evidenced by time-related reductions in the percentages of diploid (day 3-28) and tetraploid cells (day 5-28). Percentages of diploid and tetraploid cells increased on day 42 and then decreased on day 70.

Strength/Weaknesses: This study design (acute exposure followed by serial observations) is ideal for identifying the cellular target. A specific cellular marker (PCNA) was used to confirm cell proliferation. Morphological findings were confirmed at the ultrastructural level. Flow cytometric analysis was used to confirm relative changes in cell populations according to their ploidy. A variety of histologic characteristics were monitored (in table 1 of study) including not just depletion of specific cell types but also regeneration of germ cells, spermatid retention, Leydig cell hyperplasia, and changes in epididymal contents (all as recommended by Creasy, 1997 and other experts in testicular histopathology). Analysis nicely shows progression of the pathology such that spermatogonia are first depleted, followed by spermatocytes, round spermatids and elongating spermatids. Retained spermatids are not seen until a week after treatment, about the same time that exfoliated germ cells are also seen in the epididymis. Leydig cell hyperplasia is last -- apparent only at day 70 post exposure. The proportion of haploid cells rises and then falls in a manner consistent with the histological findings.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate for defining the initial cellular target as the spermatogonia and ruling out direct, immediate effects on testosterone production or Leydig cells. This helps interpret subchronic studies where some weight change in testosterone-dependent accessory organs were probably due to general toxicity and body weight loss rather than direct effects on pituitary, hypothalamus, or Leydig cells.

Table 4-10. Histological analysis in Son et al. (1999) study.

Effect	Days Adverse Effect Observed	Days Recovery Observed
Degeneration of spermatogonia in stage I-IV	1-3 and 7-70	-
Depletion of spermatogonia	3-28	42-70
Depletion of spermatocytes	5-28	42-70
Depletion of round spermatids	28-42	70
Depletion of elongate spermatids	42	70
Spermatid retention in stage IX-XII	7-28	42-70
Leydig cell hyperplasia	70	-
Oligospermia	14-70	-
Exfoliated germ cells in epididymis	7-70	-
Regeneration of germ cells	-	28-70

To obtain information about 2-BP induced effects on spermatogenesis, Wu et al. (1999b) studied the effects of 2-BP on cultured rat Leydig cells. Leydig cells were obtained from 6-week-old Sprague-Dawley rats (from Sino-British SIP-PR/BK Laboratory Animal Co.). Three replicates of cells were treated with 0, 0.01, 0.1, or 1.0 mmol/L 2-BP and 1 U human chorionic gonadotropin (hCG) hormone for 6, 12, 18, or 24 hours. A significant reduction in cell viability was noted after exposure to 1.0 mmol/L 2-

BP for 12 hours and longer and a non-significant reduction in viability occurred after exposure to 0.01-0.1 mmol/L 2-BP for 24 hours. Testosterone production decreased significantly in cells treated with 1.0 mmol/L 2-BP for 12 hours or longer. Authors concluded that 2-BP may be cytotoxic to Leydig cells *in vitro*. They noted that findings of this study were in contrast to those of Kim et al. (1996a) who found no effects on testosterone levels in humans exposed to 2-BP and Ichihara et al. (1997) who observed hyperplastic Leydig cells in rats exposed to 2-BP. The authors stated that possible reasons for inconsistencies between *in vivo* and *in vitro* studies could be metabolism of 2-BP in vivo or differences in exposure duration, doses administered, or interspecies sensitivity.

Strength/Weaknesses: Method for LC isolated should be referenced -- it's been used for many years and not developed by the authors. The rationale for concentrations used was not given – i.e. is there any relationship between the concentrations in this study and expected levels in rats after specific exposures? The discussion included reference to an unpublished study by the authors that is not appropriate or relevant.

Utility (Adequacy) for CERHR Evaluation Process: Although *in vivo* data do not suggest that Leydig cells are targeted by 2-BP, this *in vitro* study confirms that 2-BP does not directly suppress hCG -induced testosterone production at concentrations that are non-toxic to the Leydig cells. As such, the study is useful but not critical for the CERHR evaluation process.

Yu et al. (2001b) conducted a study to determine the role of apoptosis and the possible involvement of Bcl-2 family genes and the Fas signaling system in 2-BP-induced testicular toxicity. The Bcl-2 family of genes includes both pro- and anti-apoptotic proteins; the Fas signaling system transmits apoptotic signals. Male Wistar rats (12-weeks-old) were injected percutaneously with olive oil or 1350 mg/kg 2-BP (99.5% purity) in olive oil. The dosage was similar to that used in the Omura et al. 1999 study and the study authors calculated that it was equivalent to inhaling 1000 ppm for 8 hours. Seven treated groups containing 8 rats/evaluation period were injected daily for 1-5 days. The treated rats were euthanized at 12 hours following a one day treatment, 6 hours following the last day of a 1, 2, 3, or 5 day treatment period, or at 2 or 9 days following the last day of a 5 day treatment period. A control group of eight rats was euthanized 6 hours following the last day of a 5-day olive oil injection period. Six rats/group/time period were prepared for histological examination of the left testis (fixed in Bouin's) while the other 2 rats/group/time period were prepared for testicular examination by electron microscopy. Protein was extracted from the right testis for Western Blot analysis of BCL-2 and FAS proteins in 6 rats/group/time period. Data were analyzed by one-way ANOVA followed by the Tukey-Kramer multiple comparison test. Examination of Stage I and Stage VII seminiferous tubules revealed significant reductions in spermatogonia numbers following 3-5 days of treatment; no spermatogonia were detected at 2 and 9 days following the five day treatment period. A secondary reduction in pachytene spermatocyte numbers in Stage I occurred at 9 days following treatment. I*n situ* analysis of DNA fragmentation (TUNEL) conducted in 3 animals/group/time period revealed that spermatogonia were undergoing apoptosis during 2-BP treatment and pachytene spermatocytes were undergoing apoptosis nine days after the last dose was administered. DNA ladder formation in gel electrophoresis is a hallmark of apoptosis, and this assay, conducted in 1 rat/group/time period, verified the results of the TUNEL assay. Significant reductions in the expression of Bcl-2 (an anti-apoptotic protein) occurred during the first 2 days of treatment and at 9 days following treatment. Expression of Bax (a pro-apoptotic protein) was significantly increased during the first day of treatment but significantly reduced 9 days following treatment. Expression of Fas receptor was significantly upregulated during the 1st and 2nd, and 5th days of treatment and at 2 days following the last treatment. In contrast, significant reductions in Fas ligand expression were noted on the last day of treatment and 2 days following treatment. The study authors concluded that 2-BP induces apoptosis of testicular germ cells and that Bcl-2 family genes and Fas signaling system play a role in the process.

Strengths/Weaknesses: This is a high dose mechanistic study which identifies apoptosis as the process of 2-BP-induced germ cell death.

Utility (Adequacy) for CERHR Evaluation Process: At high doses of 2-BP exposure in the rat, spermatogonia undergo rapid apoptotic cell death; subsequently, after some delay, spermatocytes undergo apoptosis as well.

4.2.3 Mechanisms of Reproductive Toxicity

Park et al. (2000) tested 2-BP for estrogenic and androgenic activity in recombinant yeast expressing a human estrogen (YER) or androgen receptor (YAR) linked to a β-galactosidase reporter gene. 17β-estradiol and testosterone were used as positive controls and DMSO was the negative control. 2-BP displayed estrogenic activity at a concentration of $5x10^{-7}$ M, a level about 5,000 times higher than the concentration of 17β-estradiol that produced a significant increase in β-galactosidase activity. 2-BP did not display androgenic activity.

Strength/Weaknesses: While the utility of this recombinant yeast assay system as a universal screen for estrogens is debatable, this paper uses the method to compare a group of candidate compounds. The rationale for selecting 2-BP was apparently as an unknown that would have human exposure. Solvent and solvent control were not given.

Utility (Adequacy) for CERHR Evaluation Process: Lack of response of 2-BP in YAR (i.e. lack of androgenicity) provides indirect evidence that the mechanism of testicular toxicity of 2-BP does not involve the androgen receptor. This information is useful for risk assessment, even though none of the *in vivo* 2-BP studies point to any androgenic or antiandrogenic activity. Positive results in the YER were only seen at a very high level compared with biologically active estrogens, again suggesting that 2-BP does not act through the estrogen receptor.

4.3 Utility of Data

4.4 Summary of Reproductive Toxicity

Female Reproductive Toxicity

Reproductive effects were reported in women occupationally exposed to 2-BP. Secondary amenorrhea and increased levels of FSH and LH were observed in 16 of 25 women exposed to 2-BP in a Korean electronics plant for 4-16 months; actual exposures were not measured but were estimated to be 9.2-19.6 ppm with occasional short-term exposures of 4,141 ppm (Kim et al., 1996a; Park et al., 1997; Kim et al., 1999). Ovarian biopsies revealed fibrosis in the ovarian cortex and atrophied follicles lacking oocytes or granulosa cells, arrest of follicular development, and reduced numbers of primary follicles and corpus albicans; however the Expert Panel noted that a comparison was not made with ovaries from control women of similar ages (Koh et al., 1998). One 24-year-old woman regained menstrual cycles following estrogen-progesterone replacement therapy. A 26-year woman never regained menstrual cycles but later became pregnant and gave birth to a healthy infant. In a study conducted at a Chinese 1-BP manufacturing plant, twelve women were exposed to 0.88-16.8 ppm 2-BP (Ichihara et al., 1999). Amenorrhea was noted only in 3 older women (46-54 years) and polymenorrhea in 2 women (age 39-43 years). A regression analysis found no significant relationship between concentrations of LH, FSH, or estradiol levels and 2-BP time weighted average (TWA) or TWA x duration of exposure.

Reproductive effects observed in animal studies are similar to those observed in occupationally exposed women. Major effects noted in animal inhalation studies our outlined in Table 4-11. Nine-week inhalation studies in Wistar rats demonstrated that 2-BP targets the ovary at concentrations of ≥503 mg/m^3 (≥100 ppm) and disrupts estrous cycles at concentrations ≥1509 mg/m^3 (≥300 ppm) (Yu et al., 1999b; Kamijima et al., 1997). The primary estrous cycle effect was continual diestrus with occasional estrous, while a smaller number of rats experienced prolonged estrous with occasional diestrus. 2-BP exposure reduced the numbers of ovarian primordial, antral, and growing follicles. In the most severely affected rats, ovaries contained atretic follicles with few viable oocytes and thin granulosa cell layers and no corpora lutea. No changes in LH or FSH levels were observed in these rats. A mechanistic study suggested that 2-BP induces ovarian toxicity through apoptotic destruction of primordial follicles and their oocytes (Yu et al., 1999b). Two additional studies demonstrated reproductive toxicity in rodents, exposed to 2-BP intraperitoneally (Lim et al., 1997; Sekiguchi and Honma, 1998), but the Expert Panel noted several limitations of the studies that preclude their use in the evaluation process.

2-BP displayed estrogenic activity in an *in vitro* assay at a concentration that was about 5,000 times higher than the level of estradiol needed to produce estrogenic activity (Park et al., 2000). The Expert Panel concluded that because such a high level of 2-BP is needed to produce an effect, it is unlikely that 2-BP acts through the estrogen receptor.

Table 4–11. Summary of Reproductive Toxicity in Inhalation Studies in Female Rats

Concentration (mg/m^3)	**Exposure Regimen**	**Sex/ Species/Strain**	**Dose: Effect[a]**	**Reference**
503 1509 5031	8h/9wk; whole body	Female Wistar Rat	**503 mg/m^3:** ↓ Primordial and growing follicles. **1509 mg/m^3:** Disrupted estrous cycle; ↓ primordial, growing, and antral follicles; ↓absolute and relative uterus weight. **5031 mg/m^3:** Disrupted estrous cycle; ↓ absolute ovary weight and absolute and relative uterus weight; ↓ primordial, growing, and antral follicles; ↑atretic and cystic follicles and ↓viable oocytes, and no corpora lutea.	Kamijima et al. (1997) and Yu et al. (1999b)

↑=Increased Effect; ↓=Decreased Effect

Male Reproductive Toxicity

Two studies examined reproductive effects in males occupationally exposed to 2-BP. Azoospermia or oligospermia were observed in 6 of 8 men exposed to 2-BP in a Korean electronics plant for 16-19 months; exposures were estimated to be 9.2-19.6 ppm with occasional short-term exposures up to 4,141 ppm (Kim et al., 1996a; Park et al., 1997; Kim et al., 1999). In a study conducted at a Chinese 2-BP plant, 4 men were exposed to 0.80-5.84 ppm 2-BP (Ichihara et al., 1999). Two of the exposed men had less than 50% sperm motility but all exposed men had normal morphology and sperm counts. A regression analysis revealed no significant relationship between sperm indices or LH, FSH, or testosterone levels and 2-BP TWA or TWA x duration of exposure.

Reproductive effects in male rats exposed to 2-BP were reported in two inhalation studies; major effects in these studies are outlined in Table 4–12. Inhalation exposure of rats to ≥ 1509 mg/m^3 (≥300 ppm) for at least 9 weeks resulted in atrophy of seminiferous tubules, reductions in germ cell numbers, and hyperplastic Leydig cells (Ichihara et al., 1997; Yu et al., 2001a) in addition to reduced sperm counts and motility with increased numbers of abnormal sperm (Ichihara et al., 1997). Although inhalation is the only relevant route of human exposure, testicular lesions, sperm effects, and/or Leydig cell hyperplasia were also observed in studies with intraperitoneal, subcutaneous, or oral exposure (Yu et al., 1997; Wu et al., 1999a; Son et al., 1999). Although limited, a subcutaneous injection study in male rats treated with ≥600 mg/kg bw 2-BP for 5 weeks demonstrated reductions in mating and fertility (Wu et al., 1999a). A series of mechanistic studies identified spermatogonia as the target cells of 2-BP toxicity (Omura et al., 1997a; Omura et al., 1999; Son et al., 1999) and identified apoptosis as the mechanism of toxicity (Yu et al., 2001b). Spermatocytes also appear to be a target cell because 2-BP exposure resulted in apoptosis in spermatocytes at about nine days following treatment (Yu et al., 2001b). 2-BP did not display androgenic activity in an *in vitro* assay (Park et al., 2000).

Table 4-12. Summary of Reproductive Toxicity in Inhalation Studies in Male Rats

Concentration (mg/m^3)	Exposure Regimen	Sex/ Species/ Strain	Dose: Effect[a]	Reference
1509 5031 15,092	8h/7d/9wk; whole body (9-11 d exposure period in high dose)	Male Wistar Rat	**1509 mg/m^3:** ↓ Absolute and relative epididymides and testes weight and ↓ absolute prostate and seminal vesicles weight; ↓sperm count and motility and ↑ abnormal sperm; ↑seminiferous tubule atrophy and hyperplastic leydig cells, ↓ germ cells. **5031 mg/m^3:** ↓ Absolute and relative epididymides and testes weight and ↓ absolute prostate and seminal vesicles weight; ↓sperm count and motility, very few intact sperm remaining; ↑seminiferous tubule atrophy and hyperplastic leydig cells, ↓ germ cells. **15,092 mg/m^3:** ↓ Absolute and relative epididymides and testes weight and ↓ absolute prostate and seminal vesicles weight; ↓sperm count and motility, ↑seminiferous tubule atrophy, hyperplastic leydig cells and vacuolation of Leydig cells, ↓ germ cells.	Ichihara et al. (1997)
503 5031	8h/7d/12 wk; whole body	Wistar Rat	**Reproductive NOAEL=503 mg/m^3** **5031 mg/m^3:** Seminiferous tubule atrophy and ↓germ cells.	Yu et al. (2001)

[a]Non-reproductive Effects for male rats are summarized in Section 4
↑=Increased Effect; ↓=Decreased Effect

5.0 SUMMARIES, CONCLUSIONS, AND CRITICAL DATA NEEDS

5.1 Summary and Conclusions of Reproductive and Developmental Hazards

5.2 Summary of Human Exposure

5.3 Overall Conclusions

5.4 Critical Data Needs

NTP studies that will be conducted include 2 and 13-week studies of 1-BP, a toxicokinetics study of 2-BP and dermal uptake study of 1-BP (Morgan, 2000). No NTP developmental or reproductive toxicity studies are planned at this time (Shelby, 2000)

6.0 REFERENCES

ASTM. Standard specification for vapor-degreasing grade and general grade normal-propyl bromide, 2000.

E. A. Barnsley, T. H. Grenby and L. Young. Biochemical studies of toxic agents, the metabolism of 1- and 2-bromopropane in rats. Biochem J 100:282-288(1966).

D. M. Creasy. Evaluation of testicular toxicity in safety evaluation studies: The appropriate use of spermatogenic staging. Toxicologic Pathology 25:119-131(1997).

G. Ichihara, N. Asaeda, T. Kumazawa, Y. Tagawa, M. Kamijima, X. Yu, H. Kondo, T. Nakajima, J. Kitoh, I. J. Yu, Y. H. Moon, N. Hisanaga and Y. Takeuchi. Testicular and hematopoietic toxicity of 2-bromopropane, a substitute for ozone layer-depleting chlorofluorocarbons. Journal Of Occupational Health 39:57-63(1997).

G. Ichihara, X. Ding, X. Yu, X. Wu, M. Kamijima, S. Peng, X. Jiang and Y. Takeuchi. Occupational health survey on workers exposed to 2-bromopropane at low concentrations. Am J Ind Med 35:523-31(1999).

M. Kamijima, G. Ichihara, J. Kitoh, H. Tsukamura, K. I. Maeda, X. Yu, Z. Xie, T. Nakajima, N. Asaeda, N. Hisanaga and Y. Takeuchi. Ovarian toxicity of 2-bromopropane in the non-pregnant female rat. Journal Of Occupational Health 39:144-149(1997).

T. Kaneko, H. Y. Kim, P. Y. Wang and A. Sato. Partition Coefficients and Hepatic Metabolism in Vitro of 1-and 2-Bromopropanes. Journal Of Occupational Health 39:341-342(1997).

T. Kawai, Y. Okada, T. Odachi, S. Horiguchi, Z. Zhang, C. Moon and M. Ikeda. Diffusive sampling and biological monitoring of 2-bromopropane. Arch Environ Contam Toxicol 33:23-8(1997).

Y. Kim, K. Jung, T. Hwang, G. Jung, H. Kim, J. Park, J. Kim, D. Park, S. Park, K. Choi and Y. Moon. Hematopoietic and reproductive hazards of Korean electronic workers exposed to solvents containing 2-bromopropane [published erratum appears in Scand J Work Environ Health 1997 Feb;23(1):80]. Scand J Work Environ Health 22:387-91(1996a).

H. Y. Kim, Y. H. Chung, K. H. Yi, J. G. Kim and I. J. Yu. LC50 of 2-bromopropane. Ind Health 34:403-7(1996b).

Y. Kim, J. Park and Y. Moon. Hematopoietic and reproductive toxicity of 2-bromopropane, a recently introduced substitute for chlorofluorocarbons. Toxicology Letters 108:309-313(1999).

J. M. Koh, C. H. Kim, S. K. Hong, K. U. Lee, Y. T. Kim, O. J. Kim and G. S. Kim. Primary ovarian failure caused by a solvent containing 2-bromopropane. Eur J Endocrinol 138:554-6(1998).

C. H. Lim, S. H. Maeng, J. Y. Lee, Y. H. Chung, T. G. Kim, J. H. Park, Y. H. Moon and I. J. Yu. Effects of 2-bromopropane on the female reproductive function in Sprague-Dawley rats. Ind Health 35:278-84(1997).

S. H. Maeng and I. J. Yu. Mutagenicity of 2-bromopropane. Ind Health 35:87-95(1997).

Mallinckrodt Baker Inc. Material Safety Data Sheet for 2-Bromopropane. (1999).

D. Morgan. Personal communication from Daniel Morgan to Michael Shelby, 2000.

T. Nakajima, S. Shimodaira, G. Ichihara, N. Asaeda, T. Kumazawa, H. Iwai, I. Ichikawa, M. Kamijima, X. Yu, Z. Xie, H. Kondo and Y. Takeuchi. 2-Bromopropane-induced hypoplasia of bone marrow in male rats. Journal Of Occupational Health 39:228-233(1997a).

T. Nakajima, S. Shimodaira, G. Ichihara, N. Asaeda, T. Kumazawa, H. Iwai, I. Ichikawa, M. Kamijima, X. Yu, Z. Xie, H. Kondo and Y. Takeuchi. Histopathologic findings of bone marrow induced by 2-bromopropane in male rats. Journal of Occupational Health 39:81-82(1997b).

M. Omura, Y. Romero, M. Zhao and N. Inoue. Histopathological changes of the testis in rats caused by subcutaneous injection of 2-bromopropane. Journal Of Occupational Health 39:234-239(1997a).

M. Omura, M. Zhao, Y. Romero and N. Inoue. Toxicity of 2-bromopropane on *Spermatogonia* and *Spermatocyte*. Journal of Occupational Health 39:1-2(1997b).

M. Omura, Y. Romero, M. Zhao and N. Inoue. Histopathological evidence that spermatogonia are the target cells of 2-bromopropane. Toxicology Letters 104:19-26(1999).

OSHA and U. S. O. S. a. H. A. Directorate of Health Standards Programs. Nomination of 1-bromopropane (1-BP) and 2-bromopropane (2-bp) for testing by the National Toxicology Program, 1999.

J. S. Park, Y. Kim, D. W. Park, K. S. Choi, S. H. Park and Y. H. Moon. An outbreak of hematopoietic and reproductive disorders due to solvents containing 2-bromopropane in an electronic factory, South Korea: Epidemiological survey. Journal of Occupational Health 39:138-143(1997).

J. S. Park, B. J. Lee, K. S. Kang, J. H. Tai, J. J. Cho, M. H. Cho, T. Inoue and Y. S. Lee. Hormonal Effects of Several Chemicals in Recombinant Yeast, Mcf-7 Cells and Uterotrophic Assays in Mice. Journal Of Microbiology And Biotechnology 10:293-299(2000).

C. Reh. Submission of letters to T. Schnorr, outlining exposure levels in U.S. plants, 2000.

C. M. Reh and J. B. Nemhauser. HETA 2000-0233-2845. Indianapolis, Indiana: Trilithic, Inc., 2000.

S. Sekiguchi and T. Honma. Influence of 2-bromopropane on reproductive system--2-bromopropane inhibits forced ovulation in mice. Ind Health 36:297-299(1998).

M. Shelby. Personal communication from Michael Shelby to John Moore, 2000.

H. Y. Son, Y. B. Kim, B. H. Kang, S. W. Cho, C. S. Ha and J. K. Roh. Effects of 2-bromopropane on spermatogenesis in the Sprague-Dawley rat. Reprod Toxicol 13:179-87(1999).

X. Wu, A. S. Faqi, J. Yang, X. Ding, X. Jiang and I. Chahoud. Male reproductive toxicity and **b-**luteinizing harmone gene expression in sexually mature and immature rats exposed to 20-bromopropane. Human & Experimental Toxicology 18:683-690(1999a).

X. D. Wu, J. M. Yang, X. Y. Wu, X. C. Ding, B. Pang, X. Z. Jiang, Z. S. Ji and K. Shin. The effects of 2-bromopropane on viability and testosterone production ability of rat Leydig cells in primary culture. Biomed Environ Sci 12:43-9(1999b).

I. J. Yu, Y. H. Chung, C. H. Lim, S. H. Maeng, J. Y. Lee, H. Y. Kim, S. J. Lee, C. H. Kim, T. G. Kim, J. S. Park and Y. H. Moon. Reproductive toxicity of 2-bromopropane in Sprague Dawley rats. Scand J Work Environ Health 23:281-8(1997).

X. Yu, G. Ichihara, J. Kitoh, Z. Xie, E. Shibata, M. Kamijima, N. Asaeda, N. Hisanaga and Y. Takeuchi. Effect of inhalation exposure to 2-bromopropane on the nervous system in rats. Toxicology 135:87-93(1999a).

X. Yu, M. Kamijima, G. Ichihara, W. Li, J. Kitoh, Z. Xie, E. Shibata, N. Hisanaga and Y. Takeuchi. 2-Bromopropane causes ovarian dysfunction by damaging primordial follicles and their oocytes in female rats. Toxicol Appl Pharmacol 159:185-93(1999b).

X. Yu, G. Ichihara, J. Kitoh, Z. Xie, E. Shibata, M. Kamijima and Y. Takeuchi. Neurotoxicity of 2-bromopropane and 1-bromopropane, alternative solvents for chlorofluorocarbons. Environ Res 85:48-52.(2001a).

X. Yu, H. Kubota, R. Wang, J. Saegusa, O. Yasutake, G. Ichihara, Y. Takeuchi and N. Hasanaga. Involvement of Bcl-2 family genes and Fas signaling system in the primary and secondary male germ cells apoptosis induced by 2-bromopropane in rat. Toxicology and Applied Pharmacology 174:35-48(2001b).

W. Zhao, K. Aoki, T. Xie and J. Misumi. Electrophysiological changes induced by different doses of 1-bromopropane and 2-bromopropane. J Occup Health 41:1-7(1999).